ABSTRACT

Title of Document: THE ROLE OF HOST-PLANT HYBRIDIZATION IN HOST-ASSOCIATED POPULATION DIVERGENCE IN *PHYTOMYZA GLABRICOLA* (DIPTERA: AGROMYZIDAE).

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Phytomyza glabricola (Diptera: Agromyzidae) is a leaf-mining fly native to the eastern United States that mines two sympatric native holly species, *Ilex coriacea* and *I. glabra*. Recent work revealed significant genetic divergence between host-associated populations of flies in North and South Carolina, suggesting the populations are host forms and recent work in *Ilex* phylogenetics hint the two holly hosts may hybridize. In this work, I investigated potential ecological speciation in *P. glabricola*, hybridization in its host plants, and how the hybridization among host plants may affect gene flow between host forms of the flies.

No-choice mating trials in a greenhouse revealed reproductive isolation between host forms of *P. glabricola* and suggested female flies are capable of making oviposition mistakes resulting in adult offspring on the non-natal host. Based on these results, I used sequences of the nuclear gene EF-1α and AFLPs to genetically confirm host form status

of the flies, and identify *I. glabra* as the ancestral host. In addition, genome scans revealed several loci under divergent selection among the hosts, suggesting the flies may be undergoing ecological speciation.

To investigate the role host plants may play in the genetic divergence among flies, I first used AFLPs to confirm hybridization between *I. coriacea* and *I. glabra.* Hybridization rates differed across the geographic range of the species, which was also reflected in the morphology of the leaves. There were no general patterns, however, in the phenotypes of hybrid plants, and no single morphological trait that could reliably identify the hybrids.

Finally, I combined genetic data of the flies and the plants to determine whether hybrid plants serve as bridges or barriers for the flies. Population comparisons revealed a significant positive relationship between hybridization in the plants and gene flow in the flies, and individual comparisons indicated flies are using the hybrid plants, albeit at low levels. The results suggest hybrids could serve as bridges between parental species, helping explain how a species from a typically monophagous lineage could expand its host range.

THE ROLE OF HOST-PLANT HYBRIDIZATION IN HOST-ASSOCIATED POPULATION DIVERGENCE IN *PHYTOMYZA GLABRICOLA* (AGROMYZIDAE).

by

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DEDICATION

To my parents, Carol and Edward Byrd, for supporting me through the good and the bad.

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ABSTRACT

Speciation is the process by which taxa are split into independently evolving lineages. Where a given population or species falls on the continuum of divergence between one and two species depends on the degree of gene flow between the taxa. Reproductive isolation between taxa is one way to decrease gene flow between taxa and allow evolution to progress towards eventual speciation. In this study, I used no-choice mating trials to test for the presence of reproductive isolation between host forms of a leaf-mining fly, *Phytomyza glabricola*, on its two holly host species, *Ilex coriacea* and *I. glabra*. I found that reproductive isolation does exist between host forms in a controlled greenhouse setting. In addition, the presence of either host plant does not affect the mating success of the flies. The results indicate host forms of *P. glabricola* may be well on their way to becoming different species, although field studies are needed to validate these findings.

INTRODUCTION

How new species evolve, whether through selection or drift, in allopatry or sympatry, gradually or in spurts, has been a subject of much debate, even before the seminal works of Darwin (1858, 1859) and Wallace (1858, 1876). One large part of the debate is how to define a species (Coyne and Orr 2004). At least nine species definitions exist (reviewed in Coyne and Orr 2004), typically applied to studies to which they are most appropriate. As more research has accumulated, we have come to view speciation as a continuum with species definitions falling at different stages in the process (Harrison 1998; Dres and Mallet 2002). Where taxa fall on that continuum hinges on the degree of gene flow among diverging lineages: as gene flow decreases, lineages grow closer to species status. Because specificity to host plants can enforce reproductive isolation, a large number of studies have focused on host-associated populations (Walsh 1864; Diehl and Bush 1984; Waring et al. 1990; Abrahamson et al. 2003; Stireman et al. 2005; Dickey and Medina 2010; Barman et al. 2012), populations with varying degrees of divergence that fall in the middle of the continuum between a single and multiple species (Dres and Mallet 2002; Funk 2012).

The majority of these host-associated systems consist of host forms (Funk 1998; Funk et al. 2002; Nosil et al. 2009), populations with host-associated biological variation, but where the kind and degree of variation have not been fully examined (Funk 2012) and host races, incompletely reproductively isolated populations in sympatry that also remain distinct in the face of gene flow due to divergent selection on populations using alternate hosts (Thorpe 1930; Bush 1969; Jaenike 1981; Dres and Mallet 2002). Host forms and host races imply genetically distinct populations that are associated with different hosts,

such as in herbivorous insects (Bush 1969; Phillips and Barnes 1975; Feder et al. 1988; Brown et al. 1996; Via 1999; Abrahamson et al. 2003; Diegisser et al. 2006; Scheffer and Hawthorne 2007; Barman et al. 2012), parasites (Hoberg and Brooks 2008; Kempf et al. 2009), and parasitoids (Stireman et al. 2006; Kolaczan et al. 2009). Understanding why these host-associated populations are genetically distinct requires knowledge of the barriers to gene flow: what degree of reproductive isolation exists between host-associated groups? If it does exist, what causes the isolation? If isolation is incomplete or nonexistent, how can divergence persist in the face of gene flow? To address these questions, it is important to determine whether or not reproductive isolation does, in fact, exist. Will individuals from different host forms mate with one another and produce viable offspring?

Here, I address this most fundamental question of reproductive isolation using a newly studied host form system of a leaf-mining fly feeding on two species of holly, all of which are endemic to the eastern United States. *Phytomyza glabricola* Kulp belongs to a radiation of 14 closely related species, most of which are monophagous and all of which feed on hollies in the genus *Ilex* (Aquifoliaceae) (Kulp 1968; Scheffer and Wiegmann 2000; Lonsdale and Scheffer 2011). Unlike its congeners, *P. glabricola* feeds on two sister species of holly, *Ilex glabra* (L.) A. Gray and *Ilex coriacea* (Pursh) Chapm. *Ilex glabra's* range begins in Maine and extends south to Florida and west to northeastern Texas (Figure 1.1). *Ilex coriacea*'s range is restricted to the southern portion of *I. glabra's* range, where it is sympatric and syntopic to *I. glabra* (Scheffer 2002; JBH *pers. obs.*).

When feeding on *I. coriacea, P. glabricola* (hereafter "coriacea-flies") have a development time of approximately 9-10 months and are univoltine , whereas *P. glabricola* feeding on *I. glabra* ("glabra-flies") have a larval development time of 2-4 weeks and are multivoltine (Kulp 1968; Al-Siyabi and Shetlar 1998; Scheffer 2002; Scheffer and Hawthorne 2007). Despite these phenological differences, adult *P. glabricola* from both hosts emerge in synchrony in mid-January to mid-February (Scheffer 2002), therefore creating the opportunity for flies originally from the two host plant species to mate. Adult flies that emerge from each host do not differ morphologically in either external characters or genitalia (Scheffer 2002; Lonsdale and Scheffer 2011).

Initial work using amplified fragment length polymorphism (AFLP) frequencies revealed that fly populations from North and South Carolina show host-plant based genetic divergence (Scheffer and Hawthorne 2007). However, mitochondrial haplotypes did not cluster by host plant or location, reflecting either a lack of lineage sorting due to recent divergence or introgression via continuing gene flow (Scheffer and Hawthorne 2007). In this study, using no-choice mating trials in a full factorial design (male host, female host, and host plant(s) present) in the greenhouse, I tested same-host and cross-host mate pairs of flies to determine which fly combinations mated and produced viable offspring, and whether the success of matings depended on presence or absence of particular host plant species.

I estimated the degree of reproductive isolation by comparing the number of among-host matings (e.g., female coriacea-fly with male glabra-fly) to same-host matings (e.g., female and male coriacea-flies) producing adult offspring. This comparison

provided a coarse measure of overall reproductive isolation including prezygotic as well as extrinsic and intrinsic postzygotic barriers encompassing mating, oviposition, larval development, and successful emergence of adult flies. Flies must survive all of these stages in order to be able to pass their genes on to the next generation; therefore all are required for successful gene flow. If coriacea-flies and glabra-flies are completely reproductively isolated, I expected no successful among-host mate pairs to produce viable offspring. If there is partial reproductive isolation between coriacea-flies and glabra-flies, I expected some among-host mate pairs to be successful, but significantly less than same-host mate pairs. Finally, if there is no reproductive isolation between coriacea-flies and glabra-flies, I expected no difference in the success rate of among-host and same-host mate pairs.

Varying host plant species in the mating chambers allowed me to assess the importance of the physical presence of the host plant species in mate choice and mating success. If flies have a mating preference based on presence of the natal host plant, I expected more successful matings when the natal host was present than when the natal host was absent. In addition, I included trials with both host species and observed the presence/absence of leaf-mines (successful larval development) and from which mines adult flies emerge on each host plant species. I then used differences in the presence of leaf-mines, from which leaf-mines adult flies emerged, and the time taken to develop within the leaf-mine, to identify the role of host plant species on the success of mate pairs and the basis of differences in development time.

METHODS

Collections

Flies were collected in January and February of 2006 from Croatan National Forest in North Carolina and Francis Marion National Forest in South Carolina (Figure 1.1). Leaves containing well-developed leaf-mines were removed from host plants and placed into plastic bags labeled for site and host plant species. Abundance of leaf-mines and rates of parasitism varied between locations, leading to unequal sample sizes among populations. Pupae were dissected from mines and placed individually in 0.5 mL Eppendorf tubes and stored in a moist chamber until the emergence of adults.

Mating trials

No-choice mating experiments were performed in modified 16 ounce plastic cups surrounding small propagated host plants in the greenhouse (Figure 1.2). A total of 107 trials were conducted using every combination of male fly and female fly (from *I. coriacea* or *I. glabra*) placed in mating chambers with either *I. glabra*, *I. coriacea*, or both host plants present (Table 1.1, Figure 1.2). As flies only live a few days in the greenhouse, mate pairs were generated as soon as a male and female fly eclosed from the same location. The host plant(s) on which they were tested was randomized. Each trial was observed twice a day to note formation of leaf-mines and the emergence of adults from pupae. Dead parental flies were removed from the cup, placed in 100% ethanol, and stored at -80˚C. Trials were considered unsuccessful if no leaf-mine was formed after three months.

A Pearson's chi-squared contingency test was used to determine if there was a significant difference in the success rate between same-host and among-host mating

trials, allowing me to determine whether different host forms of *P. glabricola* were capable of producing adults that could potentially allow introgression of alleles among host forms. Hence, a mating trial was considered successful if the mate pair produced offspring that eventually emerged as an adult from at least one of the mines inside the mating chamber. First, I compared the number of successful and unsuccessful trials for same-host versus among-host mate pairs to test for overall reproductive isolation. Next, I compared the number of successful and unsuccessful trials in presence and absence of the natal host plant species to test whether the natal host species is required for mating success. Last, I compared the number of successful and unsuccessful trials in presence versus absence of the non-natal host to test whether the non-natal host prevents successful mating. Tests were performed in the statistical package R (v2.7.2, R Development Core Team, 2010). P-values were computed using a Monte Carlo test (Hope, 1968) with 10^7 replicates to compensate for a potential lack of power due to small sample sizes.

To address whether differences in development time in the wild are only under genetic control, means and standard errors were calculated for development time on each host. In addition, a 2-sample heteroscedastic t-test was conducted in R to test whether development time differed between offspring from different parental combinations.

RESULTS

Only 12 of the 107 trials successfully resulted in adult offspring, all of which were same-host trials (Table 1.1). Despite the low number of successful matings, significantly more same-host trials were successful than among-host trials ($X^2 = 7.44$,

$p < 0.01$). Because no among-host trials produced offspring, the remaining results refer to same-host trials only.

Host plant species presence had no effect on mating success. The presence of the natal host did not appear to increase mating success in either coriacea-flies ($X^2 = 4.8081$, $p = 0.06977$) or glabra-flies (glabra-flies: $X^2 = 0.0845$, $p = 1$; Table 1.2). In addition, the non-natal host did not decrease mating success (coriacea-flies: $X^2 = 0.4444$, $p = 0.6561$; glabra-flies: $X^2 = 1.712$, $p = 0.3127$; Table 1.2). Interestingly, adult offspring emerged from both host plant species for both coriacea-fly and glabra-fly same-host matings. Offspring emerged from coriacea-fly same-host trials on *I. coriacea* alone as well as trials with both host plant species present (Table 1.1). For the latter, adults emerged from leaf-mines on *I. coriacea* as well as from *I. glabra*. Offspring from glabra-fly same-host trials emerged from trials on *I. coriacea* alone and trials on *I. glabra* alone (Table 1.1).

Finally, all offspring emerged from each host plant species within two months of the start of the trial. Offspring produced from coriacea-fly mate pairs took 45 ± 2.0 days to emerge whereas flies from glabra-fly same-host mate pairs emerged in 54 ± 5.4 days. The time to emergence did not significantly differ between coriacea-fly and glabra-fly same-host crosses ($t = 1.55$, $df = 6.35$, $p = 0.17$).

DISCUSSION

When studying speciation, it is important to determine the degree of gene flow between potentially reproducing populations. In this study, I demonstrated the presence of reproductive isolation between host-associated populations of *P. glabricola* on its host plants, *I. coriacea* and *I. glabra*. I found host plant species presence had no effect on

mating success. My results suggest host forms of *P. glabricola* may be well on their way to becoming distinct species.

The lack of viable offspring from any among-host mate pairs suggests the presence of prezygotic or postzygotic barriers to gene flow. No mating behavior was observed and I could not detect oviposition unless a leaf-mine formed, so I was unable to separate premating isolation from among-host inviability. The apparent reproductive barriers indicate that genetic signatures of gene flow (Scheffer & Hawthorne 2007, Chapter 2) are more likely due to incomplete lineage sorting than to ongoing gene flow.

There were a low number of successful trials, possibly due to performing the experiments in the greenhouse rather than the natural environment. Conditions in the greenhouse were optimal for plant growth (temperature, light, and water controlled with fertilizer), and are likely different from conditions in their natural pocosin habitat (sandy soil over peat, acidic, low in nutrients such as nitrogen and phosphorus, and often poorly drained although seldom standing water; Smith et al. 1956; Wilbur and Christensen 1983; Richardson 1991; Mitchell et al. 1995). The increased nutrient levels in the green house could have changed important traits such as plant volatiles, physiological chemistry, and secondary metabolites (Kainulainen et al. 1996; Gaston et al. 2004; Scutareanu and Loxdale 2006; Nell et al. 2009; Olson et al. 2009; Winter and Rostas 2010; Ibrahim et al. 2011), all of which could affect the willingness of flies to mate and oviposit (Feder et al. 1995; Nishida et al. 1996; Gouinguene and Stadler 2005; Joyce et al. 2008; Cook et al. 2011) and the ability of offspring to complete their life cycle (Potter 1992; Melo et al. 2006). For example, Diptera are known to use pheromones derived from nutrition sources (Tillman et al. 1999) for signaling during courtship (reviewed in Wicker-Thomas 2007).

Changes to the chemistry of their host could change the pheromone composition, preventing the completion of copulation. In addition, the small containers may have interfered with visual courtship displays commonly found in flies, including agromyzids (Ota and Nishida 1966; Carriere and McNeil 1988). Two species of *Phytomyza* have also been found to use substrate-borne courtship songs (Kanmiya 2006); the vibrations from fans and other equipment in the greenhouse could disrupt such acoustic signaling. Future work should focus on the rates of same-host and across-host matings in natural conditions.

Flies from the same host plant successfully mated and success did not depend on which host plant species was present, suggesting host plant presence does not affect mating success (either as an attractant or a deterrent). In addition, offspring from these mate pairs were able to emerge from both *I. coriacea* and *I. glabra*, regardless of the parents' natal host. Therefore, it is possible that females could make oviposition "mistakes", laying eggs on non-natal hosts, and if the offspring can survive on the non-natal host, as suggested here, these mistakes could lead to gene flow among host forms.

Again, the lack of a difference could be due to the greenhouse setting. Changes in plant chemistry and general substrate could affect mating preferences. Furthermore, these were no-choice trials, so females could have oviposited on the non-natal host out of necessity, whereas in normal conditions, they would not. Field work is needed to determine whether host plants affect mating success, females oviposit on the non-natal host, and if larvae and pupae of flies can survive in wild populations.

Unexpectedly, all offspring of successful mate-pairs emerged within two months, irrespective of what host plant they emerged from. Flies from *I. coriacea* typically take

nine months to develop in the wild, as opposed to two months for flies from *I. glabra* (Scheffer 2002). The reduction in development time suggests there is at least some environmental component to the flies' rate of development on each host plant species. Insect development can depend on how well host plants are defended: insects tend to have more generations on plants that are less well defended (Hunter and McNeil 1997; Steinbauer et al. 2004; van Asch and Visser 2007), and host plant quality can influence the induction of diapause (Hunter and McNeil 1997; Ito 2003; Ishihara and Ohgushi 2006; Ito and Saito 2006; Takagi and Miyashita 2008). Foliar nitrogen content is known to vary with soil nutrients (Marschner 1995) and can directly affect the growth rate of phytophagous insects (White 1993; Cornelissen and Stiling 2006). The addition of fertilizer in the greenhouse could explain more rapid development if *I. glabra* is better able to obtain nitrogen than *I. coriacea* in natural populations.

The mechanisms underlying reproductive isolation in these flies warrants further investigation. I do not yet know the specific mating behavior of these flies, so I may have missed key features important for mating success such as space for flies to move in, additional mates to choose from, day length, or external temperatures. In addition, changes in nutrient content could affect plant volatiles and nutrition, which could play a role in oviposition choice and mating behavior. Future work should focus on replicating natural conditions to determine whether or not complete reproductive isolation exists between host forms of *P. glabricola*.

I have now established that reproductive isolation exists between host forms of *P. glabricola*, so I can begin to investigate what ecological, behavioral, and/or genetic factors serve as barriers to gene flow in this system. It is important to use recently

diverged populations and species to study these barriers, as the original factors causing divergence may disappear over time (Coyne and Orr 2004). Using populations in the 'grey area' of species status will allow us to determine what elements are the most important drivers of speciation, and therefore understand how the great biodiversity we see today originally arose.

In conclusion, my study suggests populations of *P. glabricola* are closer to the species end of the speciation continuum between populations and species. My mating trials indicate a large degree of reproductive isolation exists among populations of *P. glabricola* on its two host plant species, corresponding to previous molecular work demonstrating significant genetic divergence between the host forms (Scheffer and Hawthorne 2007). I found that female flies will oviposit on both host plant species and offspring are capable of surviving on the parental non-natal host in greenhouse conditions. In addition, I found no evidence that flies must mate in the presence of their natal host or the absence of the non-natal host, indicating migration may be possible between host forms. However, because no cross-host mating pairs produced viable offspring, I have no indication that migration will result in gene flow between host forms of the flies.

Table 1.1. Mating trials of *Phytomyza glabricola* on its host plants, *Ilex glabra* and *I. coriacea*. Trials were considered successful if the flies mated, the female oviposited eggs, and the offspring successfully emerged as adults.

Male fly	Female fly	Host-plant species present	# Successful Trials	Total # of Trials
Glabra	Glabra	Glabra	3	12
Glabra	Glabra	Coriacea	2	12
Glabra	Glabra	Both	0	11
Glabra	Coriacea	Glabra	0	5
Glabra	Coriacea	Coriacea	0	5
Glabra	Coriacea	Both	0	4
Coriacea	Glabra	Glabra	0	8
Coriacea	Glabra	Coriacea	0	8
Coriacea	Glabra	Both	0	8
Coriacea	Coriacea	Glabra	0	12
Coriacea	Coriacea	Coriacea	3	11
Coriacea	Coriacea	Both	4	11
		Total	12	107

Table 1.2. Comparison of mating trials of *Phytomyza glabricola* in presence versus absence of the natal and non-natal host plant species. Trials were considered successful if the flies mated, the female oviposited eggs, and the offspring successfully emerged as adults.

		Natal present	Natal absent	Non-natal present	Non-natal absent
Coriacea-Coriacea mate pairs	Successful	7	0	4	3
	Unsuccessful	15	12	19	8
Glabra-Glabra mate pairs	Successful	3	2	2	3
	Unsuccessful	20	10	21	9

Figure 1.1: Endemic range of the host plants, *Ilex coriacea* and *I. glabra* with collection sites labeled.

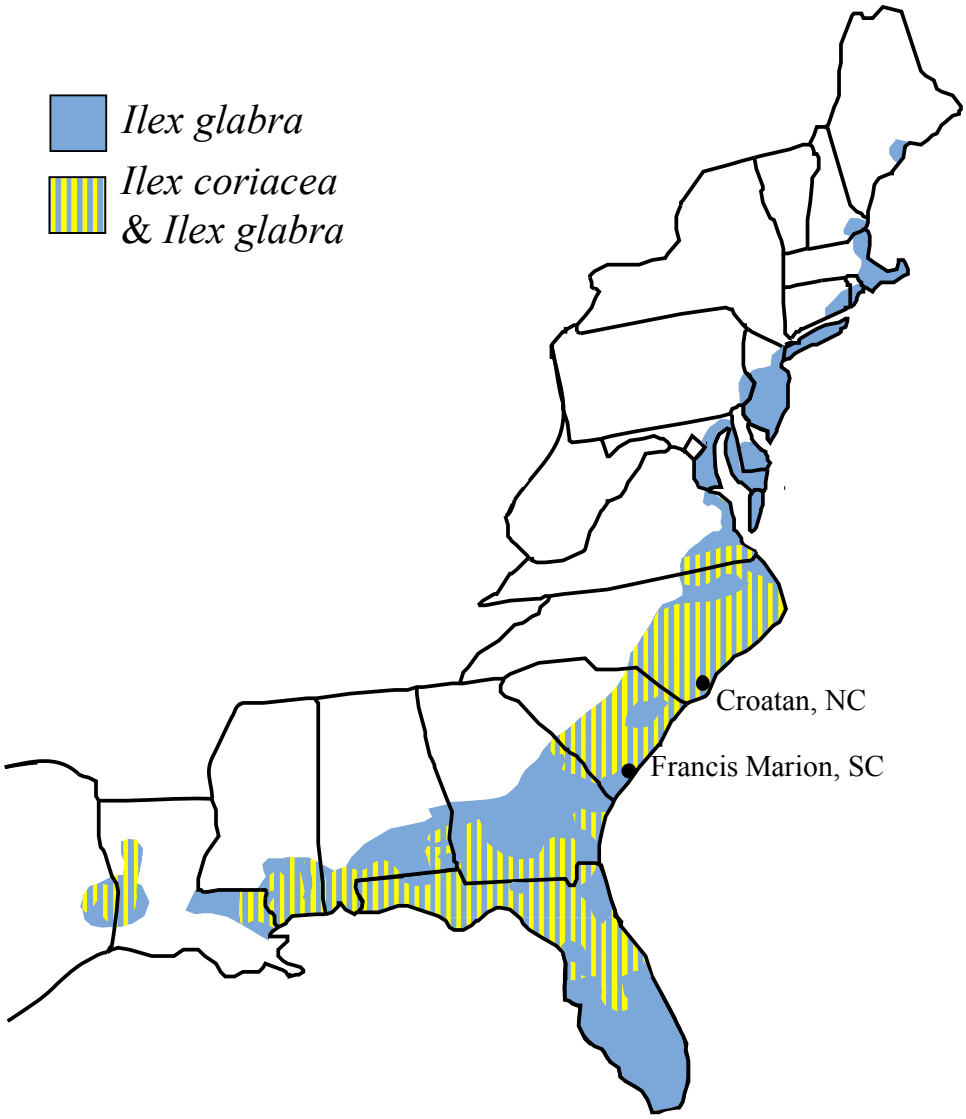
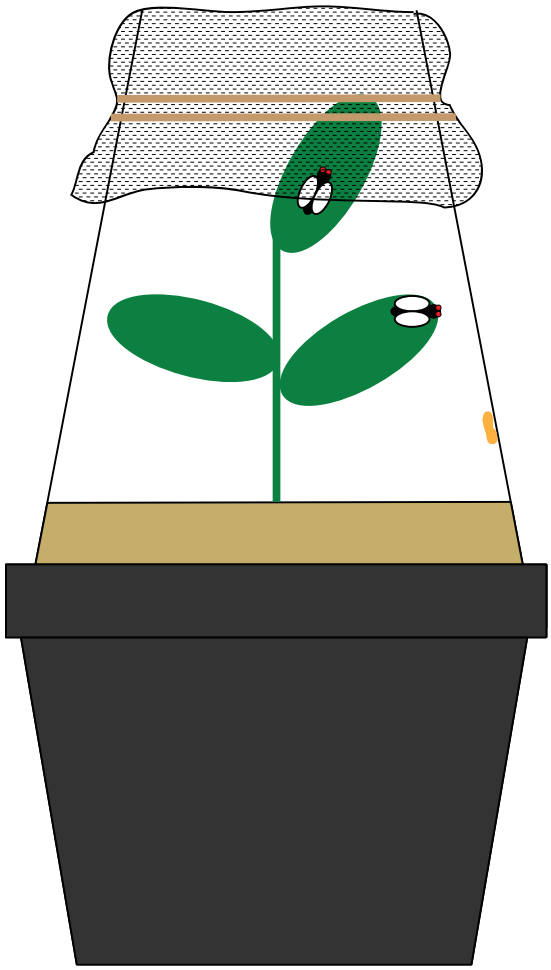


Figure 1.2: Diagram of mating chamber. A piece of foam surrounds the base of the plant in its pot, sealing the bottom portion of the cup. Fine mesh was held over the cup with a rubber band. Honey was placed on the side of the cup so that flies had a food source.



CHAPTER 2: EVIDENCE FOR ECOLOGICAL SPECIATION IN THE HOLLY LEAF-MINER, *PHYTOMYZA GLABRICOLA* (DIPTERA: AGROMYZIDAE)

ABSTRACT

Evolutionary radiations have been well documented in plants and insects, but we have yet to determine the relative impact of genetic drift and natural selection underlying these radiations. If the radiations are adaptive, the diversity of species could be due to ecological speciation in these lineages. Agromyzid flies are known to have repeated host-associated radiations, so I take advantage of previously identified host forms of *P. glabricola* associated with *Ilex coriacea* and *I. glabra* to test whether the species undergoing ecological speciation. Using AFLPs and nuclear sequence data, I found a geographic mosaic of genetic divergence between host forms across the range of these flies. Flies on *I. glabra* are multivoltine whereas flies on *I. coriacea* are univoltine, and voltinism is at least partially controlled by the environment, suggesting plant-mediated genetic divergence could lead to host race formation without the evolution of host preference. The data also suggest the flies expanded from *I. glabra* to *I. coriacea* and are now experiencing divergent selection. Genome scans revealed several loci under divergent selection in multiple populations of these flies. It appears *P. glabricola* is in the process of ecological speciation, suggesting ecological speciation could be at least partially responsible for host-associated radiations in these flies.

INTRODUCTION

Understanding the evolution of biotic diversity is one of the primary aims of biology. Phytophagous insects are known to be extremely diverse, making up over 25% of the total terrestrial biodiversity (Strong et al. 1984; Price 2008). Much of the diversity was generated by radiations of phytophagous insects onto a number of host plant taxa, particularly angiosperms (Mitter et al. 1988; Farrell 1998; Winkler and Mitter 2008). The wide variety of chemical and morphological defenses of plants combined with a number of plant modules (e.g., leaves, stems, flowers, and fruits) provide many adaptive zones (Simpson 1949, 1953) in which phytophagous insects can specialize (Ehrlich and Raven 1964; Price 2008).

Ehrlich and Raven (1964) described how evolutionary radiations of plant lineages could result from the evolution of novel defensive chemistry, followed by evolutionary radiations of phytophagous insects from reciprocal changes to adjust to that chemistry (i.e., 'escape and radiate'). Evolution of key innovations, such as the ability to digest plant defensive chemicals combined with dispersal into a new habitat (e.g. a host plant range expansion) provide ecological opportunities needed for adaptive radiations (Simpson 1949, 1953; Mitter et al. 1991; Schluter 2000; Yoder et al. 2010). If insects mate on their host plant, specialization to a host plant species can result in reproductive isolation and can eventually lead to speciation (Ehrlich and Raven 1964; Wheat et al. 2007; Janz and Nylin 2008).

Although patterns of host-associated radiations have been well-documented (Mitter et al. 1988; Farrell 1998; Winkler and Mitter 2008; Yoder et al. 2010), our understanding of the speciation processes and host specialization that give rise to these

patterns remains contentious (Coyne and Orr 2004). Darwin first connected speciation with adaptive divergence to different habitats (Darwin 1859). Conceptual models shifted to allopatry with neutral accumulated changes (Mayr 1963; Nosil 2008) followed by sympatric models where fitness and reproduction were associated with habitat preference (Bush 1969; Felsenstein 1981; Rice and Hostert 1993; Hawthorne and Via 2001; Schluter 2001; Via 2001; Feder et al. 2005). More recently, studies of speciation have shifted away from a focus on geographic distribution towards an emphasis on ecologically-based adaptive divergence causing reproductive isolation in either allopatry or sympatry, termed 'ecological speciation' (Futuyma and Moreno 1988; Rundle et al. 2000; Schluter 2000).

In phytophagous insects, adaptation to different host plants can decrease gene flow between host-associated populations of insects, especially if the insects reproduce on the host (Smith 1966; Diehl and Bush 1984; Schluter 2001; Turelli et al. 2001; Via 2002). Specific host-associated systems such as host forms (Funk 1998; Funk et al. 2002), populations with an unknown kind and/or degree of host-associated biological variation (Funk 2012) and host races (Thorpe 1930; Bush 1969; Jaenike 1981; Dres and Mallet 2002) demonstrate intermediate steps in speciation, representing the evolution of ecological divergence. Still, ecological divergence is not synonymous with speciation, and we have yet to determine the relative impact of divergent selection versus genetic drift on whether or not speciation proceeds to completion. To determine whether ecological speciation could be responsible for radiations of phytophagous insects on host plants, we need to focus currently diverging or recently evolved taxa within adaptive radiations and determine whether ecological speciation can account for the divergence.

The Agromyzidae (leaf-mining flies) show considerable evidence of repeated host-associated radiations (Spencer 1990; Scheffer and Wiegmann 2000; Winkler et al. 2009b). The genus *Phytomyza* is the largest Agromyzid genus, and is comprised of a large number of host-associated radiations primarily associated with the Ranunculaceae and families within the Asteridae (Winkler et al. 2009a; Winkler et al. 2009b). *Phytomyza glabricola* Kulp, a species endemic to the eastern United States, belongs to a radiation of 14 closely related species, all of which feed on hollies in the genus *Ilex* (Aquifoliaceae) and most of which are monophagous (Kulp 1968; Scheffer and Wiegmann 2000; Lonsdale and Scheffer 2011). Unlike its monophagous congeners, *P. glabricola* feeds on two sister species of holly, *Ilex glabra* (L.) A. Gray and *Ilex coriacea* (Pursh) Chapm, both of which are also endemic to the eastern United States (Selbach-Schnadelbach et al. 2009; Manen et al. 2010).

Ilex glabra and *I. coriacea* are found in baygall and pocosin habitats in the coastal plains of the eastern United States (Caughey 1945; Richardson 1983, 1991; Brooks et al. 1993). *Ilex glabra* is present from Maine south to Florida and west to northeastern Texas (Figure 2.1). *Ilex coriacea* is sympatric with *I. glabra* (Scheffer 2002), but it has a much smaller distribution, limited to the southern portion of *I. glabra's* range. It also has a patchier distribution than *I. glabra*, likely due to lower tolerance of dry conditions (Mohlenbrock 1976; Brooks et al. 1993). Where sympatric, the plants are often also syntopic, with leaves from one plant commonly in contact with leaves of the other species. In addition, the two species likely hybridize in nature (Robert K. Godfrey Herbarium 2012, Specimens 000016759-000016766) Hybridization is not surprising considering *Ilex* species are often very genetically similar to one another (Cuenoud et al.

2000; Setoguchi and Watanabe 2000; Manen et al. 2002; Manen 2004; Manen et al. 2010), as evidenced by the many ornamental *Ilex* cultivars that have been generated by interspecific hybridization (Galle 1997).

In the field, when feeding on *I. glabra, P. glabricola* (hereafter "glabra-flies") have a development time of approximately 2-4 weeks and are multivoltine, whereas *P. glabricola* feeding on *I. coriacea* ("coriacea-flies") have a development time of 9-10 months and are univoltine (Kulp 1968; Al-Siyabi and Shetlar 1998; Scheffer 2002; Scheffer and Hawthorne 2007). Despite these phenological differences, adult *P. glabricola* from each host emerge in synchrony in mid-January to mid-February (Scheffer 2002). Adult flies that emerge from each host do not differ morphologically in either external characters or genitalia (Scheffer 2002; Lonsdale and Scheffer 2011). On greenhouse grown plants, female flies will oviposit on the non-natal host, and the offspring can develop into adult flies (Scheffer *pers. comm.*; Chapter 1). Mating trials also indicate the presence of reproductive isolation between flies from the two host plant species (Chapter 1).

Initial work revealed that fly populations from North and South Carolina show host plant-based genetic divergence based on amplified fragment length polymorphism (AFLP) frequencies (Scheffer and Hawthorne 2007). However, mitochondrial haplotypes did not cluster by host plant or location, reflecting either a lack of lineage sorting due to recent divergence or introgression via continuing gene flow (Scheffer and Hawthorne 2007).

Whether divergence exists throughout the range of these insects and their host plants has not been examined and could differ for several reasons. The host plant ranges

do not fully coincide, so there may be different degrees of divergence in locations supporting only *I. glabra*. The host plant range spans a very wide latitudinal gradient possibly altering intrinsic and extrinsic factors such as developmental patterns and natural enemy abundances. In addition, as mentioned above, the host plants likely hybridize in nature, and initial morphological observations suggest hybridization rates differ among locations (see Chapter 3). Because hybridization could produce plants with mixed traits, it could change the distribution of insects on the host plants in different locations, potentially affecting the degree of gene flow in flies among host plant species (see Chapter 4).

In this study, I first asked is the degree of genetic divergence across the natural range of *P. glabricola* similar to the results of Scheffer and Hawthorne (2007)? I used DNA sequence data from the nuclear protein-coding gene Elongation Factor-1α (EF-1α) as well as AFLP data to test for host-associated genetic divergence from populations spread across the sympatric range of the host plant species. I also used this data to examine the amount and direction of gene flow between host forms of *P. glabricola* by identifying migrants and offspring of cross-host matings.

If host-associated radiations of agromyzids, particularly in *Phytomyza* species feeding on *Ilex*, are a result of host expansions followed by ecological speciation, I expected to find a pattern of a host range expansion where flies from one host plant species are ancestral to flies from the other species, and genetic signatures of divergent natural selection. First, to determine the direction of the initial host range expansion, I estimated diversity and genetic structure using the EF-1α dataset. I expected more genetic variation and older haplotypes in flies from the ancestral host plant (Harrison 1991;

Brown et al. 1996), whereas there should be no difference in the diversity and relative age of haplotypes if both host forms of flies arose at the same time, such as from an additional host.

Finally, I asked whether the genetic divergence is due to natural selection or genetic drift associated with vicariance. I used genome scans of AFLPs to detect genetic patterns of divergent selection among genomes of coriacea-flies and glabra-flies then tested for linkage disequilibrium between outliers. If divergent selection reduced gene flow between host forms, the genomic architecture of selected loci, such as physical linkage or sex-chromosome linkage, would increase the likelihood of eventual ecological speciation in *P. glabricola.*

MATERIALS AND METHODS

Collections

Flies were collected in January and February of 2006 from Croatan National Forest in North Carolina and Francis Marion National Forest in South Carolina, and again in 2007 with additional samples from Cape Henlopen State Park, DE, the Great Dismal Swamp National Wildlife Refuge, VA, Crooked River State Park, GA, Etoniah Creek State Forest, FL, and Apalachicola National Forest, FL (Figure 2.1). *Ilex glabra* was found at every collection site; however *I. coriacea* was absent from the most northern sites (NY, NJ, DE, MD) which are outside the plant's geographic range, and from the GA and Archibold, FL sites. Leaves containing well-developed leaf-mines, and visible larvae, were removed from host plants and placed into plastic bags labeled for site and host plant species. Pupae were dissected from mines and placed individually in 0.5 mL Eppendorf

tubes and stored in a moist chamber until adults emerged. Adult flies were placed in 100% ethanol and stored at -80˚C.

AFLPs

Genomic DNA was extracted from 183 individual flies (96 coriacea-flies and 87 glabra-flies) following the animal tissue protocol of the Qiagen DNeasy kit (Qiagen, Valencia, CA). DNA concentrations were standardized to 12.5 ng/μL. AFLP constructs were assembled in a single-tube reaction by mixing 30.0 μL of genomic DNA, 5.0 μL 10 X NEBuffer 3 [100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 1 mM dithiothreitol (DTT)] (New England Biolabs, Ipswich, MA), 0.5 μL 100X bovine serum albumen (BSA), 5.0 μL 10mM ATP, 5 units *Pst*I, 5 units *Eco*RI, 100 units T4 DNA ligase (Genscript, Piscataway, NJ), and 1 μL each of 5 μM double-stranded *Eco*RI and *Pst*I adapters (Hawthorne 2001). The reactions were incubated at 37 C for 5 hours and then 80 C for 20 minutes. Each reaction was then diluted 1:10 with ultrapure H_2O and stored at -20 C.

A two-step amplification was used (Vos et al. 1995): the preamplification step used one selective base on each primer (*Eco*RI-A and *Pst*I-A) in a 10 μL reaction [1.5 mM $MgCl_2$, 0.125 mM dNTPs, and 0.5 units Taq DNA polymerase (Genscript, Piscataway, NJ) combined with 0.25 μM primers and 2.0 μL of template DNA]. The reaction was cycled 21 times for 30 sec at 95 C, 1 min at 56 C, and 1 min 72 C with an additional extension period of 5.5 min at 72 C. Preamplification products were diluted 1:40 with ultrapure H_2O and stored at -20 C. The selective amplification was performed using the same 10 μL cocktail, but with a fluorescein amidite (FAM)-labeled primer with additional selective bases in place of *Eco*RI-A (Table 2.1). A touchdown-PCR was used

starting with an annealing temperature of 65 C which was decreased by 1 C for 8 rounds of amplification, followed by 22 rounds of amplification at an annealing temperature of 58 C, and an additional extension period of 7 min at 72 C. PCR products were separated with an ABI 3730 DNA Analyzer (Applied Biosystems, Carlsbad, CA) using MapMarker X-Rhodamine (ROX) labeled 1000bp ladder (BioVentures, Murfreesboro, TN).

Electropherograms were scored using GENEMAPPER v.3.7 (Applied Biosystems, Carlsbad, CA). Fragments between 76 and 800 base pairs were first scored using the automated procedure and secondarily checked by eye. To measure the repeatability of peaks, six individuals were repeated across plates and an additional ten individuals replicated within each plate. Negative controls (H_2O template) were included at every step of the process. A genotyping error rate was estimated as the ratio of electropherogram peak mismatches among the replicates to the total number of replicated markers (Pompanon et al. 2005). Loci with peak mismatches among repeated samples were removed from the analysis as were loci occurring at the same sizes as peaks observed in the negative controls. Mismatches were not equally distributed among loci: some loci had only a single individual with a mismatch whereas others showed mismatches in a large number of individuals. Therefore, the percentage of loci removed due to mismatches was much higher than the overall genotyping error rate. Finally, because a significant negative correlation of fragment frequency and fragment size may be caused by excessive homoplasy, I estimated that correlation using AFLPSURV (Vekemans et al. 2002).

Nuclear sequence data

A 910-bp DNA fragment of the nuclear protein-coding gene Elongation Factor-1α (EF-1α) was amplified from genomic DNA of 236 flies (122 coriacea-flies and 114 glabra-flies) collected in 2006 and 2007, and 46 flies (25 coriacea-flies and 21 glabra-flies) from a previous study (Scheffer and Hawthorne 2007) using the primers found in Table 2.1. A standard amplification protocol was used to amplify the fragments, with initial denaturation at 95°C for 2 min followed by 12 cycles of 92°C for 15 s, 56°C for 30 s, and 70°C for 1 min 30 s, then 32 cycles of 92 °C for 10 s, 55 °C for 15 s, and 72 °C for 1 min 30 s, with a final extension at 72 °C for 10 min. PCR products were purified using either the QIAquick PCR purification kit or the QIAquick gel extraction kit (Qiagen, Inc.). Purified PCR product was used in sequencing reactions with BigDye sequencing kits (Applied Biosystems, Foster City, CA) and the products generated using an ABI-3130 automated sequencer (Applied Biosystems). Diploid sequencing was conducted using nested primers to ensure overlap of at least two amplifications for each sample (Table 2.1). Sequence contigs were assembled and aligned using CODONCODE ALIGNER (v.2.0 CodonCode Corp., Dedham, MA). Heterozygous states were identified as dual peaks. The reading frame of the final consensus sequence was determined by comparison with EF-1α100E and EF-1α48D in *Drosophila melanogaster*. Allelic phase for EF-1α sequences was reconstructed using the program CVHAPLOT (v.2.01 Huang et al. 2008; Huang and Zhang 2010). CVHAPLOT runs the sequences through several phase-determining programs, each of which has a different algorithm for phase-determination. The resulting haplotypes are then compared among analyses to check for consensus between programs. CVHAPLOT was run with the entire data set, then with flies from each

host plant separately. Separating flies from each host plant gave a better consensus among programs, therefore those results were used. Taking a conservative approach, only samples with agreement in 5 or more programs were included in the following analyses (Huang et al. 2008; Huang and Zhang 2010).

Geographic scale of host plant associated genetic divergence

Host plant associated genetic differentiation was estimated for the entire data set as well as within geographic locations using both AFLP markers and EF-1α sequence data. For AFLPs, 5000 permutations were run to calculate and test the significance of F_{ST} using AFLPSURV (v.1.0 Vekemans et al. 2002). For EF-1α sequence data, Φ_{ST} (Excoffier et al. 1992) was estimated using ARLEQUIN (v.3.5 Excoffier et al. 2005). F_{ST} (and Φ_{ST}) for within-host comparisons among locations were calculated only if at least five individuals were present in a population on a single host plant species; for among-host comparisons, locations were only included if at least five individuals were present on each host plant species.

I took two additional approaches to measuring the genetic divergence among flies collected from different plant species and locations: an analysis of molecular variance was performed using a permutational MANOVA via the ADONIS function from the VEGAN package (Oksanen et al. 2010) in the statistical package R (v 2.11.1, 2010), and using a clustering method that required no *a priori* hypotheses of substructure using the AFLP data. The distance matrix for EF-1α was calculated using the F84 model of nucleotide substitution (Kishino and Hasegawa 1989; Felsenstein and Churchill 1996) in DNADIST, part of the PHYLIP package (v.3.69 Felsenstein 2005); Jaccard distances were calculated using the AFLPs because they are based only on the shared presence of peaks.

ADONIS models were constructed to test the effects of host plant source and collection site location on the genetic structure of flies from all locations and to test the effects of collection year using only the locations common to all years collected. ADONIS models were also constructed to test the effects of sex of the fly on genetic structure of the flies using AFLP data. Models were run with host plant source nested within location. Models for each analysis were first run with all interactions then interactions were sequentially removed if non-significant. Significance was based on 5000 permutations producing pseudo-F ratios.

Second, I performed nonmetric multidimensional scaling (NMDS) on pairwise Jaccard genetic distance estimates between individual genotypes to visualize the data in two dimensions. Using NMDS, I am also able to estimate the correlation of a series of explanatory variables, including host plant source, sex, and year, with genetic distances among individuals. The ordination was generated using the function METAMDS, also part of the VEGAN package in R, and the magnitudes of variance attributable to the categorical explanatory variables were tested using a goodness of fit statistic based on 5000 permutations of environmental variables on the ordination data using the function ENVFIT in R.

Estimation of cross-host plant oviposition and gene flow

Individuals collected from one host plant that carry a multilocus genotype that predominates in the other may signal a cross-host plant oviposition in which a female deposits an egg into the host plant from which neither she nor her mate emerged. These individuals provide an estimate of the oviposition infidelity of females for their natal host plant. Hybrids or more advanced backcrosses between host plant-specific genotypes

indicate cross-host plant gene flow. Hybrid AFLP genotypes (F1 and backcrosses) were identified using NEWHYBRIDS (Anderson and Thompson 2002; Anderson 2008). The choice of prior had no effect on the overall likelihood of the results, so calculations were run without individual-specific assumptions using 'Jeffreys-like' prior for the mixing proportion and a uniform prior for allele frequency. Simulations were run with a burn-in period of 8×10^4 iterations followed by 1.5×10^6 sweeps for sampling from the posterior distribution. Ancestry was determined based on three thresholds: the category with the highest posterior probability for each individual, or with a threshold of 90% or 75% probability of being a parental form with the rest considered "introgressed" individuals.

Identification of ancestral and novel host plants

Because a recent divergence of flies from ancestral to novel host plants may result in reduced genetic diversity in leaf-miner populations on the novel host plant, I compared the diversity of EF-1α haplotypes and AFLP genotypes of flies from the two host plant species to infer which is ancestral and which is novel. For EF-1α sequence data, the number of haplotypes (H), polymorphic sites (p),haplotype diversity (H_d, Nei 1987), and nucleotide diversity (π, Tajima 1983) were estimated using ARLEQUIN (v.3.5 Excoffier et al. 2005). The number of singleton haplotypes (S_n) and nucleotide diversity (π, Tajima 1983) were estimated using and DNASP (v.5.1 Librado and Rozas 2009). For AFLPs, Nei's genetic diversity (H_J) and the average gene diversity within populations (H_S) were calculated using AFLPSURV (v.1.0 Vekemans et al. 2002).

The topology of a haplotype network can also provide clues to the relative ages of haplotypes. Haplotypes that represent nodes that are relatively internal versus at the tips of a network and haplotypes that are more abundant and geographically widespread are

likely to be older (Donnelly and Tavare 1986; Golding 1987; Crandall and Templeton 1993). To visualize the relationships among EF-1α haplotypes found in flies from each host plant I generated a median-joining network using NETWORK (v.4.5.1.6 Bandelt et al. 1999; Polzin and Daneschmand 2003). The network was rooted using EF-1α sequences from three closely related species: *P. ilicis*, *P. ilicicola*, and *P. ditmani* to further inform my inference of the relative ages of haplotypes from different host plants (Winkler et al. 2009b).

Host-associated divergent selection

Migration, mutation, drift, and inbreeding are expected to affect all loci in a genome in a similar fashion. In contrast, selection should have locus specific effects: selected sites should show lower genetic diversity and increased genetic differentiation among populations with contrasting environments relative to the rest of the genome (Beaumont and Balding 2004; Egan et al. 2008; Nosil et al. 2009). I used genome scans to identify AFLP loci whose divergence exceeds that expected by genetic drift associated processes alone to infer the action of selection in causing genetic divergence among flies using the two host plants. I used two methods to detect outliers in several geographic locations to gain confidence in my results by rejecting false positives that are identified in single comparisons and with different analyses (Luikart et al. 2003; Stinchcombe and Hoekstra 2008). First, I identified AFLP loci using a hierarchical-Bayesian approach in DFDIST (Beaumont and Balding 2004), and then I directly asked which loci were likely diverged by selection using BAYESCAN (v.1.0 Foll and Gaggiotti 2008). To generate a seed for creation of a null distribution of F_{ST} in DFDIST, a trimmed mean F_{ST} for each population was estimated that excludes the highest and lowest 30% of locus-specific F_{ST}

estimates in the AFLP data set (Weir and Cockerham 1984; Zhivotovsky 1999; Bonin et al. 2006). Using this seed, DFDIST creates a distribution of F_{ST} for individual loci given assumptions of neutrality and independent evolution of loci (loose linkage). Thresholds identifying exceptionally divergent or constrained allele frequencies found in comparisons of different populations can then be determined using that distribution. Here, loci with an F_{ST} in the upper 95% and 99% confidence intervals of the simulated distributions were labeled "outliers" and are candidates for divergent selection. DFDIST was run using the total data set, then for NC, SC, and eastern Florida populations, as loci repeatedly identified in more than one location are considered especially robust (Campbell and Bernatchez 2004; Bonin et al. 2006; Egan et al. 2008; Nosil et al. 2008; Hohenlohe et al. 2010).

Unlike DFDIST, BAYESCAN estimates the posterior probability of a given locus under two models, evolving neutrally or under selection, using a reversible MCMC approach in which F_{IS} is allowed to vary between 0 and 1. BAYESCAN was run starting with a burn-in period of 20 pilot runs, each with a length of 10^4 iterations. The burn-in was followed by 40 thinning intervals each with 10^4 iterations for a total of 400 000 iterations. Outliers were identified as loci with posterior probabilities of being under selection at the 'strong' (0.91-0.97), 'very strong' (0.97-0.99), and 'decisive' (>0.99) levels.

Genomic architecture of divergent loci

Recent studies in flies have found divergent loci located within chromosomal inversions (Noor et al. 2001; Coluzzi et al. 2002; Feder et al. 2003; Brown et al. 2004; Ayala and Coluzzi 2005), which are expected to show differences faster than normally

recombining regions. Inversions are common in *Phytomyza* (Block 1969a, 1974), so I estimated linkage disequilibrium among host-associated divergent loci to determine whether the loci were clustered with one another suggesting close physical linkage. Because my calculations of LD assume the populations are in Hardy-Weinberg equilibrium, LD was estimated separately for flies from each host plant. Separate analyses also prevented confounding loci in LD because of selection or shared history with those in LD because of physical linkage. Estimates of allele frequencies and LD between AFLP markers were performed as described by Hill (1974) using the statistical package R (v2.11.1, 2010; Appendix A; code available upon request). A chi-square test with one degree of freedom was used as an approximation of the likelihood ratio of LD to no LD to infer significance of LD comparisons (Hill 1974). I used a correction for multiple comparisons based on false discovery rates (Pike 2011) for all tests of LD to account for multiple non-independent comparisons.

Sex chromosomes are often associated with speciation because they are expected to show differences in F_{ST} faster than other parts of the genome (Muller 1942; Charlesworth et al. 1987; Haldane 1992). Agromyzids have been shown to have an XX/XY sex chromosome system (Block 1969a, b, 1974, 1975a, b, 1976), allowing me to compare allele frequencies of male and female flies within hosts to predict whether host-associated outliers may be located on the X- or Y-chromosome. I estimated allele frequencies and variation in these estimates for each host-associated locus for male and female coriacea-flies, and male and female glabra-flies, using the same iterative model used for estimating LD (Hill 1974). In addition, I estimated allele frequencies for each host-associated locus in male coriacea-flies and male glabra-flies using a haploid model.

If a host-associated outlier was located on the Y-chromosome, I expected an absence of peaks in female flies and the presence of peaks in male flies at that locus. If a host-associated outlier is located on the X-chromosome, I expected the allele frequency estimates of that locus for female coriacea-flies to be more similar to estimated allele frequencies of male coriacea-flies calculated using a haploid model than those using a diploid model. I used a t-test with one degree of freedom to compare estimates of female allele frequencies to estimates of male allele frequencies using the haploid model and again for the diploid model.

RESULTS

A total of 656 AFLP markers were scored from 183 flies giving an initial error rate of 5.5% (Table 2.2, Appendix B). An additional 258 markers were removed due to discrepancies across repeated samples. No plate effect was found, however linkage disequilibrium analyses resulted in patterns of linked markers of the same size from different primer pairs, indicating non-specific primer binding occurred in the samples. There was a higher probability of non-specific primer binding in this study due to one primer being used in all primer-pair combinations. Where identified, all but one locus were discarded to eliminate replicated markers, resulting in a total of 305 markers. Finally, using a more conservative cutoff than the typical 5%, any markers where only one individual contained the rarer allele were discarded, giving a final total of 265 markers. The size range of the AFLP markers was 78-792bp, and 92% had a fragment size above 200 bp. The Pearson correlation coefficient between fragment sizes and fragment frequencies was not significant ($r = -0.0137$, $p = 0.82310$), indicating a low risk of homoplasy due to small fragment sizes (Vekemans et al. 2002).

A total of 308 flies were genotyped for a 910 bp sequence of EF-1α, resulting in 27 SNPs (Table 2.2, Appendix B). Only 279 individuals (145 coriacea-flies and 134 glabra-flies) had 5 or more votes in the consensus analysis of CVHAPLOT, reducing the dataset to 75 distinct genotypes and 43 haplotypes with 22 polymorphic sites (Table 2.3). The translated sequence matched that of EF-1α48D (95% match), and all polymorphic sites were in third codon positions (Figure 2.2). Both coriacea-flies and glabra-flies showed signs of recombination between haplotypes (minimum number of recombination events based on the four allele approach, Hudson and Kaplan 1985; Table 2.4; Fu 1997).

Geographic scale of host plant associated genetic divergence

The mean allele frequencies did not differ between sample years for AFLPs ($F = 1.17435$, $df = 1$, $p=0.226$, Table 2.5) nor EF-1α ($F = 2.2811$, $df = 1$, $p=0.168$, Table 2.6), therefore data were combined among years. Results from the ADONIS function (Tables 2.5 and 2.6) and analyses using F_{ST} were similar, therefore only F_{ST} is given here. Significant genetic divergence was found among flies from different host plant species using both AFLPs ($F_{ST:}$ 0.1247, $p < 0.0005$) and EF-1α ($\Phi_{ST:}$ 0.50744, $p < 0.001$; Table 2.7). Host-associated differences were also significant in all three geographic locations, but varied in magnitude among locations (Table 2.7). Estimates of Φ_{ST} using EF-1α increased in a southerly direction (Table 2.7). The opposite was seen in AFLPs: the most southern population in eastern Florida had the lowest F_{ST} whereas the northern populations (North and South Carolina) had higher values of $F_{ST.}$ There were significant, but smaller, differences in allele frequencies among flies from different locations within the same host plant species for AFLPs (coriacea-flies: F_{ST}: 0.0482, $p = 0.0182$ glabra-

flies: F_{ST}: 0.0178, p = 0.0146; Table 2.7) and EF-1α (coriacea-flies: Φ_{ST}: 0.02844, p = 0.00880; glabra-flies: Φ_{ST}: 0.01263, p = 0.09677; Table 2.7).

Using the AFLP data, the 183 individuals formed four distinct groups on the first two NMDS axes, corresponding to host plant and sex of the fly (Figures 2.3, 2.4). The Kruskal's stress for the final ordination was 22.9%. Both host plant and sex were significantly correlated with the ordination of the AFLP data (host plant: $R^2 = 0.4965$, p = 0.0002, Figure 2.3a; sex: $R^2 = 0.3123$, p = 0.0002, Figure 2.3b). Visually, flies from each host plant clearly separated along the first axis, and males and females separated along the second axis (Figure 2.4). The differentiation among the sex of the flies likely represents good coverage of the genome, and is likely driven by distances among individuals associated with sex chromosomes and genes that influence sex formation. Location was also significantly associated with the NMDS ordination, (location: $R^2 = 0.0804$, p = 0.0024), but only if the two locations with only glabra-flies included; if they were removed, location was no longer significant (location: $R^2 = 0.0272$, p = 0.3243).

The results from NEWHYBRIDS indicated low rates of gene flow between host plants. Using the majority-rules threshold, none of the flies were identified as F_1 hybrids, but 18 individuals were classified as backcrosses with coriacea-flies and one individual as a backcross with glabra-flies (Appendix C). Using the 90% threshold, 36 individuals were identified as introgressed individuals in a primarily coriacea-fly genome, and eight as introgressed individuals in a primarily glabra-fly genome (Appendix C). Using the 75% threshold, those numbers dropped to 21 and 2, respectively. Regardless of the

threshold used, the introgression patterns indicate bidirectional gene flow with asymmetric movement of glabra-fly alleles to coriacea-fly genomes.

Identification of ancestral and novel host plants

The mean genetic variability of EF-1α is lower for coriacea-flies than glabra-flies regardless of the measure used (Table 2.4). Coriacea-flies have less haplotype and nucleotide diversity, and fewer average pairwise differences than glabra-flies. In addition, there were 14 haplotypes and only one singleton found in coriacea-flies, whereas glabra-flies had more than twice as many haplotypes (36) and 13 singletons (Figure 2.5). There were 7 haplotypes shared between the flies on the different host plants. The most common haplotype (h13) was found in 126 of the 135 coriacea-flies but only one glabra-fly (Figure 2.5). Unlike host plant, there were no geographic patterns in the network (Figure 2.6).

AFLPs revealed a different pattern. Estimates of genetic diversity were fairly similar among host forms. Coriacea-flies had slightly more polymorphic loci than glabra-flies (Table 2.8), but glabra-flies had slightly higher values for Nei's genetic diversity and Nei's H_S (Table 2.8). Dividing the AFLPs into outlier and non-outlier loci did not change this result. Glabra-flies had slightly higher genetic diversity than coriacea-flies with both outlier and non-outlier loci (Table 2.8)

The majority of the haplotypes found in coriacea-flies are found in a cluster distal to the most similar haplotypes found in glabra-flies. This cluster is distinguished by alternative alleles of a single SNP (snp4; Figure 2.5). The SNP was nearly a fixed difference between host forms, however 2.8% of coriacea-flies are homozygous for the glabra-fly allele, 19.3% of coriacea-flies were heterozygous, and 0.7% of glabra-flies

were homozygous for the coriacea-fly allele. No glabra-flies were heterozygous at this position.

Host-associated divergent selection

Genome scans in DFDIST testing for divergent selection between populations on each host plant in each location indicated 32 loci (12.5%) had F_{ST} higher than the 95th percentile of the simulation results in at least one comparison (Table 2.9; Appendix D). Of those, 24 (9.3%) were significant outliers in multiple locations. When all populations were combined, 15 (5.7%) outliers were significant among host plants (Figure 2.7; Tables 2.9, 2.10). All but two (loci 200 and 238) of the 15 loci found in the combined comparison were also significant using BAYESCAN (Table 2.10), and all but locus 238 were significant in multiple independent comparisons. These two loci were the closest outliers to the cutoff in DFDIST, so they had a lower likelihood in general of being outliers (Figure 2.7).

The values of Φ_{ST} in EF-1 α were very similar to the F_{ST} estimates using only outlier AFLP loci (Table 2.7). Given the high F_{ST}, but the lack of non-synonymous changes in the DNA sequence, the data suggest EF-1α is likely near a locus under divergent selection. When EF-1α is added to the AFLP outliers present in multiple independent comparisons, there appear to be 15 loci showing signs of divergent selection among host plants in these flies.

When I examined the distribution of peaks among populations of coriacea-flies and glabra-flies, no strong patterns emerged (Table 2.11). Both coriacea-flies and glabra-flies had five fixed or nearly-fixed loci, only one of which was shared between them (locus 118): it had nearly a fixed presence in coriacea-flies and a nearly-fixed absence in

glabra-flies (Table 2.11). Where populations were nearly fixed, the individuals with the minority allele were typically found in populations from North and South Carolina, where I collected over two years and had larger sample sizes (Tables 2.2, 2.11). Of the outliers with lower support, locus 200 had a fixed absence in glabra-flies, likely driving its identification as an outlier, but was only at mid-level frequencies in coriacea-flies, reducing its likelihood of experiencing divergent selection (Table 2.11). Locus 238 was at higher frequencies in coriacea-flies than glabra-flies, but the difference was not enough to be identified using smaller sample sizes in location comparisons or using the Bayesian approach.

Among samples of coriacea-flies collected from different locations, 13 loci (6.4%) were identified as significant outliers in DFDIST (Tables 2.9, 2.10). None of those markers were outliers in more than one independent comparison and only one was identified in BAYESCAN (locus 144 within coriacea-flies; Table 2.10; Appendix D). Two of the 16 outlier loci were also identified as host-associated outliers (locus 70 and locus 72; Table 2.10). Upon further examination, the outlier status appeared to be driven by coriacea-flies in eastern Florida (Table 2.11, Appendix D). These populations lack a fixed absence in locus 70 and a nearly fixed presence in locus 72 found in in all other populations (Table 2.11).

Glabra-flies had fewer location-specific outliers: 10 loci (5.1%) were identified at the 95% level in DFDIST (Tables 2.9, 2.10), none of which were significant in BAYESCAN. Two of the among-location outliers identified in glabra-flies were also outliers in coriacea-flies in DFDIST (locus 167 and locus 226; Table 2.10). Within-host location-associated divergence for locus 167 was driven by genetic differences in eastern Florida

for both coriacea-flies and glabra-flies (Table 2.11; Appendix D). Locus 226 was more complicated: differences existed between both northern populations and Florida, and between eastern and western Florida populations within coriacea-flies, but differences in glabra-flies were driven by the population in Delaware (Table 2.11; Appendix D).

Genomic architecture of divergent loci

Two groups of loci were identified in LD among coriacea-flies (70-72 and 115-118-246) and one pair of loci in LD among glabra-flies (242-255; Figure 2.7). Loci 70 and 72 were also identified as host-associated outliers as well as location-associated outliers within coriacea-flies largely due to genetic differences in the population from eastern Florida (Tables 2.10, 2.11; Appendix D). These differences could potentially explain why these loci appear to be in LD within the coriacea-flies as well.

I did not detect LD among the remaining host-associated outlier loci. However, six host-associated outliers were in LD with sex-related outliers. In coriacea-flies, host-associated outliers 94 and 115 were in LD with sex-associated locus 188, and host-associated loci 72 and 213 were in LD with sex-associated locus 113. In addition, host-associated locus 238 was in LD with the 8 sex-associated loci (20, 32, 41, 125, 132, 249, 251, and 261). Glabra-flies only had one host-associated locus (231) in LD with a sex-associated locus (188).

My estimates of male and female allele frequencies gave no evidence of among-host outliers on the Y-chromosome. All of but one of these loci either had peaks in multiple females, or if no peaks in females, also had no peaks in the males for that population (Table 2.12). The only locus with no peaks in females and peaks in males was

locus 231 in glabra-flies; however coriacea-fly females did have peaks (Table 2.12). There was no significant interaction between host plant and sex of the fly, so I have no reason to expect locus 231 to be on a sex chromosome in one host-associated population but not the other.

I also did not see strong evidence of the presence of host-associated outliers on the X-chromosome. For most loci, estimates of allele frequencies using a diploid model for male flies more closely resembled female allele frequencies than estimates using a haploid model (Tables 2.13, 2.14). Three loci had significant differences between female allele frequency estimates and those using the diploid male model, but not with estimates using the haploid male model (loci 72, 213, and 238; Table 2.13). Two of those loci, markers 72 and 213, were closer to the diploid male model in glabra-flies but not coriacea-flies (Table 2.13). Locus 238 had more similar allele frequency estimates between females and haploid males in both coriacea-flies and glabra-flies; however, the haploid model was also close to significantly different in both cases (Table 2.13).

To clarify whether 238 could be located on the X-chromosome, I examined allele frequency estimates of the sex-associated outliers with females, a haploid male model, and a diploid male model (Table 2.14). All of the sex-associated outliers in LD with host-associated locus 238 showed a pattern of being on a Y-chromosome, not an X-chromosome, making it unlikely locus 238 is located on a sex chromosome in these flies.

DISCUSSION

The results of this study show *Phytomyza glabricola* may be in the process of ecological speciation among its two host plant species, *Ilex coriacea* and *I. glabra*. Host-associated genetic divergence is present across the geographic range of *P. glabricola*,

although the magnitude varies among locations and among genetic markers. I found evidence of contemporary gene flow, indicating host forms of the flies are likely not yet different species despite previous evidence of reproductive isolation (Chapter 1). The flies likely expanded from *I. glabra* to *I. coriacea*, but enough time has passed to eliminate much of the demographic signature of a host range expansion from the genome of coriacea-flies. Instead, genetic divergence appears to be primarily driven by natural selection, as expected if the flies are in the process of ecological speciation. However, I did not detect physical linkage among AFLP outlier loci, nor did the loci appear to be on sex chromosomes, two features often tied to an increased probability of eventual speciation. Still, I cannot completely eliminate the potential presence of inversions or sex-linkage due to the low resolution of AFLP loci in this study.

Geographic scale of host-associated genetic divergence

Host-associated genetic structure exists across the range of *P. glabricola*, supporting the previous identification of coriacea-flies and glabra-flies as host forms (Scheffer and Hawthorne 2007; Funk 2012). Divergence among host plants is much larger than divergence among locations within a given host, meaning coriacea-flies from Florida are more genetically similar to coriacea-flies from Delaware than they are to glabra-flies from Florida. In addition, the degree of genetic divergence among host forms varies among locations.

The variation in the degree of host-associated genetic divergence could be due to environmental differences between geographic locations. Higher temperatures and increased daylight hours in the south could increase developmental rates of flies in these locations. Flies on *I. glabra* experience multiple generations in a year, whereas flies on

I. coriacea have only a single generation (Scheffer 2002). The additional generations of flies on *I. glabra* could give more chances for adaptive traits to arise via recombination and mutation in glabra-flies, and selection could more efficiently eliminate slightly deleterious alleles, especially if alleles allowing coriacea-flies to use *I. coriacea* are maladaptive on *I. glabra*, increasing the degree of divergent selection on glabra-flies. If so, plant-driven temporal differences without allochronic isolation could result in increased genetic divergence among host forms. A host range expansion from *I. glabra* to *I. coriacea* could have immediately resulted in genetic divergence among populations on each host plant species, without a need for preference of a particular host or differences in performance.

Environmental differences could also indirectly impact the populations of flies by changing the relative abundances of their host plants. *Ilex glabra* tolerates a wider range of temperatures than *I. coriacea*, and is also more tolerant of dry conditions (Mohlenbrock 1976; Brooks et al. 1993). Locations with less rain fall and cooler temperatures may have a higher relative abundance of *I. glabra*. Much like the increase in the number of generations, an increased population size of glabra-flies could increase the genetic variation in the population, allowing selection to more efficiently eliminate deleterious alleles. However, the abundance of flies is not necessarily tied to the abundance of the host species. Individual *I. coriacea* plants tend to have a higher density of leaf-mines than do *I. glabra* (JBH, S.J. Scheffer *pers. obs.*), even though a given location typically has more *I. glabra*, so the two could balance out to even the relative population sizes of host forms of the flies. It is also possible the geographic variation in estimates of F_{ST} could be a sampling effect as sample sizes from eastern Florida were

smaller than those in North and South Carolina, and too small in the other locations to have any confidence in estimates of F_{ST}.

Direction of host range expansion

There are three probable scenarios to explain how *P. glabricola* has diverged between *I. coriacea* and *I. glabra*. Either *I. coriacea* is the ancestral host and flies expanded to *I. glabra*, vice versa, or the ancestral flies were originally on a different plant species that they no longer use, and expanded onto *I. coriacea* and *I. glabra* from that third ancestral host. *Phytomyza glabricola* is not found on host plants other than *I. coriacea* and *I. glabra*, and most in the clade are monophagous, therefore a shift from one current host to the other appears more likely than a shift to both from an additional species. The combination of the haplotype network and genetic divergence present in host forms points towards *I. glabra* as the ancestral host.

Haplotypes that are relatively internal in a network are likely to be older than haplotypes at the tips of the network (Donnelly and Tavare 1986; Golding 1987; Crandall and Templeton 1993). Closely related taxa used as outgroups for the network were most closely related to primarily glabra-fly haplotypes (Figure 2.5). In addition, the haplotypes most characteristic of coriacea-flies were found within an offshoot of the main network, analogous to a nested clade within a phylogram. The topology suggests either that a subset of flies from *I. glabra* colonized the novel host plant, bringing along only a small fraction of the ancestral genetic diversity (Harrison 1991; Brown et al. 1996), or natural selection is reducing the genetic variation of either EF-1α, or a locus closely linked to it, in coriacea-flies but not glabra-flies.

Coriacea-flies also had less genetic variation in EF-1α than glabra-flies, but roughly equal genomic variation based on AFLPs. There are two main reasons to expect less genetic diversity in coriacea-flies than glabra-flies: either the flies expanded from *I. glabra* to *I. coriacea* and coriacea-flies are not yet in mutation selection balance, or coriacea-flies are adapting to a novel environment. Given the high Φ_{ST} among host forms (0.51662, Table 2.7) and the presence of only synonymous substitutions, it appears EF-1α may be closely linked to a locus under divergent selection. In addition, if the lowered variation in EF-1α were due to a founder event following a host range expansion, I would expect the AFLPs to show reduced diversity in coriacea-flies as well, as drift should affect all loci similarly (Cavalli-Sforza 1966; Lewontin and Krakauer 1973; Vitalis et al. 2001). Thus, if the flies expanded from *I. glabra* to *I. coriacea*, it was long enough ago that additional genetic variation has arisen throughout the genome of coriacea-flies.

The differences in development time on each host could also affect the genetic diversity of EF-1α in each host form. If EF-1α is near a locus under divergent selection, I would expect less genetic diversity in glabra-flies due to the increased effect of selection compounded over multiple generations, which does not match the pattern seen. Instead, it appears that the strength of selection on coriacea-flies is stronger than the increased effect of selection due to multiple generations. However, multiple generations could also allow for increased recombination between EF-1α and the selected locus, potentially increasing diversity in EF-1α in glabra-flies, which I cannot eliminate as a possibility with these data.

Identifying the ancestral and novel host plant will allow me to investigate factors that may have driven the initial host range expansion. Enemy-free space is a strong

possibility (Denno et al. 1990; Gratton and Welter 1999; Murphy 2004), as populations of *P. glabricola* experience parasitism rates of 50 to 100% (JBH *pers. obs.*) with a trend of higher parasitism on *I. glabra* than *I. coriacea*. Flies could also have expanded to a new host plant species to escape competition on the ancestral host plant or gain a new resource. However, I find many *I. coriacea* and *I. glabra* with no leaf-mines on them, and plants do not seem to be saturated with leaf-mines, suggesting a lack of strong competition. More work is needed to elucidate what selection pressures may differ between the host plants, and how those affect genetic divergence between the fly populations.

Asymmetrical gene flow

Very low rates of gene flow were found among populations of coriacea-flies and glabra-flies. No fixed differences in either AFLPs or EF-1α were found between host forms of *P. glabricola*. In addition, none of the individual flies were identified as F_1 hybrids; but, a number of individuals were identified as backcrosses, indicating either F_1 hybrids are present at a low frequency within these populations, or the putative backcrosses are presenting unsorted ancestral polymorphism. Most of the individuals identified as having introgression predominantly had a coriacea-fly genetic background.

The asymmetry of gene flow could have several explanations. Flies on *I. glabra* are multivoltine whereas flies on *I. coriacea* are univoltine (Scheffer 2002). If voltinism has at least a partial environmental component linked to the host plant (Chapter 1), F_1 and backcrossed flies on *I. glabra* will have multiple generations in which they will likely mate back to the parental glabra-flies, potentially masking bidirectional gene flow by eliminating easily identifiable glabra-fly backcrosses. The additional generations would

also allow selection to more efficiently remove slightly deleterious alleles. If selection in the additional generations results in increased specialization to *I. glabra*, any preference that evolves could potentially reduce the willingness or ability of glabra-flies to use the alternate host plant, *I. coriacea*. Future work should sample flies from the second generation on *I. glabra* to determine whether or not F_1 and backcrossed individuals are present and eliminated in future generations, or instead, if gene flow is primarily unidirectional from *I. glabra* into *I. coriacea*.

On the other hand, asymmetrical gene flow may not be directly influenced by the host plant. Expansion to a novel host plant species is associated with changes in host acceptance, host use, and mate choice (Janz and Nylin 2008). If coriacea-flies are less choosy, they may be more likely to migrate to another host plant and may also be less choosy about mates. Previous work in *Drosophila* species demonstrated asymmetrical mating between ancestral and founding populations where female choose mates based on specific mating behavior (Kaneshiro 1976; Ohta 1978). Males in the founding population putatively lose parts of the polygenic mating ritual via drift and cannot mate with ancestral females, whereas females from the founding population will mate with ancestral males, and potentially as time goes on, with novel males (Kaneshiro 1980). If the same is true for coriacea-flies, coriacea-females may mate with glabra-males and males of mixed ancestry, but glabra-females may not, resulting in a greater number of backcrosses to coriacea-flies.

Host-associated divergent selection

Ecological speciation is defined as ecologically-based *adaptive* divergence. To investigate whether the genetic divergence I found between coriacea-flies and glabra-flies

shows signatures of divergent selection, I used genome scans to identify several AFLP loci with a higher F_{ST} between host-associated populations than expected due to drift processes alone. All but two of these loci were also identified as outliers in multiple independent population comparisons and/or using multiple methods of identification, lending support to their outlier status.

The arrangement of presences and absences within an outlier locus among populations pointed to divergent selection on both hosts rather than directional selection on one host and balancing selection on the other. If the latter was the case, I would have expected more outlier loci with near-fixed and fixed differences in the population experiencing directional selection, but the number of outlier loci with fixed and near-fixed differences was equal among host forms.

EF-1α was also likely near a locus under divergent selection. The high estimates of Φ_{ST} among host forms were more similar to F_{ST} estimates using AFLP outliers than to estimates using non-outlier loci across the geographic range of *P. glabricola*. If EF-1a is physically linked to a locus under divergent selection, the increased number of generations of glabra-flies could explain why the patterns of divergence seen in EF-1a among locations (increasing F_{ST} in a southerly direction) differs from the patterns seen with AFLPs (lower F_{ST} in Florida relative to North and South Carolina); AFLPs should represent both selection and demographic effects, whereas EF-1a could just represent the strength of divergent selection. If southern populations of glabra-flies have a greater number of generations than northern populations, the increased effects of selection near EF-1a could lead to the increased host-associated genetic divergence in southern locations.

Fewer AFLP outlier loci were found when comparing among locations within host plant than among host plants, and only one locus was found to be significant in multiple independent comparisons within DFDIST, indicating divergent selection is much stronger between host plants than local adaptation (Table 2.10). Most location-associated outliers within both glabra-flies and coriacea-flies were due to differences between populations in eastern Florida and the other populations. These differences could be due to environmental conditions in Florida. For example, winter diapause is terminated by high temperatures in a congeneric, *P. chaerophylli* (Frey 1991). If the same is true for *P. glabricola*, flies in southern populations could experience earlier diapause, and in the case of glabra-flies, potentially more generations in southern populations. On the other hand, if differences were due to temperature, I would expect to see similar differences associated with the populations from western Florida in coriacea-flies, but this was not the case. More work is needed to determine what is causing flies from eastern Florida to differ from the other populations.

Genomic architecture of divergent loci

The genomic architecture of host-associated outliers both reflects the past evolution of genetic divergence and will affect how rapidly genetic divergence will continue to evolve between host forms of *P. glabricola*, therefore affecting the likelihood of speciation in these lineages. LD will accumulate among markers in genomic regions experiencing reduced recombination, such as within chromosomal inversions (*reviewed in* Hoffmann and Rieseberg 2008) or in regions containing loci under especially strong selection (Beaumont and Balding 2004; Via and West 2008; Nosil et al. 2009). To examine whether 'genomic islands' exist in *P. glabricola*, I looked for LD between host-

associated outliers in coriacea-flies and glabra-flies separately to make a very coarse inference of the genomic architecture of the divergence.

I found little evidence for physical linkage among host-associated outliers in these flies. Host-associated outliers found to be in LD within coriacea-flies were not the same as outliers in LD in glabra-flies. Coriacea-flies and glabra-flies could have different adaptive genes, in addition to different alleles, potentially explaining the loci in LD found with coriacea-flies and not glabra-flies, and vice versa (Hawthorne and Via 2001). The differences could also appear to be in LD due to chance. Estimates of LD using dominant markers require the assumption of Hardy Weinberg Equilibrium (HWE), and the outliers clearly do not meet that assumption (Bonin et al. 2004). In addition, by testing for LD within a given host form, outlier loci close to fixation in that host form would have little or no variation with which to detect linkage disequilibrium. However, if host forms were combined, I could not separate LD due to divergent selection (as expected with the outlier loci) from physical linkage.

Host-associated outliers found to have LD in one host form, but not the other, could be due to genomic rearrangements in coriacea-flies relative to glabra-flies. Chromosomal inversions have been associated with speciation (*reviewed in* Hoffmann and Rieseberg 2008), but I expect host-associated outliers within an inversion should be more likely to show up as in LD in both populations, due to a reduced likelihood of recombination in inversions (Hoffmann and Rieseberg 2008; Feder and Nosil 2009). However, given the course genomic resolution of AFLPs and the lack of a linkage map or a sequenced genome on which to map the host-associated outliers, I cannot say for sure that chromosomal rearrangements cannot be associated with the genomic distribution of

outliers in these flies. Although evidence of LD among host-associated outliers would have indicated potential islands of speciation, not finding significant LD does not mean markers are not linked or within an inversion. The degree of coverage by AFLP loci here is not enough to negate the potential presence of physical linkage.

Sex chromosomes are also expected to show differences in F_{ST} faster than other parts of the genome due to a smaller effective population size as a result of Haldane's rule (Muller 1942; Haldane 1992; Wu and Davis 1993; Turelli and Orr 1995; Wu et al. 1996) and the large X-effect (Charlesworth et al. 1987; Coyne and Orr 1989; Coyne 1992; Masly and Presgraves 2007), and are consequently often associated with speciation. I did not see convincing evidence of X- or Y-linkage of the host-associated outliers. I cannot say for sure that host-associated outliers in *P. glabricola* are not located on the sex chromosomes because I do not have a linkage map or sequenced genome on which to map the markers. So-called 'speciation genes' have been associated with sex chromosomes in other systems (Wittbrodt et al. 1989; Barbash et al. 2000; Phadnis and Orr 2009), so further work is needed to determine whether or not it could also be the case in *P. glabricola*.

Conclusions

Host forms of *Phytomyza glabricola* show a geographic mosaic of genetic divergence on their host plants, *Ilex coriacea* and *I. glabra*. Patterns of genetic divergence associated with differences in voltinism on each host plant suggest genetic divergence could arise among host-associated populations without the evolution of host preference. Differences in development time also likely manifest themselves in asymmetrical bidirectional gene flow, in this case with primarily glabra-fly alleles

introgressing into the coriacea-fly background, giving the appearance of unidirectional gene flow. However, I could not eliminate the possibility of unidirectional gene flow associated with the host range expansion of glabra-flies onto *I. coriacea*, which may have resulted in less fidelity in host acceptance, host use, and mate choice in coriacea-flies.

I detected evidence for divergent selection among host forms of *P. glabricola* associated with both EF-1α and fifteen AFLP outlier loci. Although I would expect stronger selection on coriacea-flies to adapt to the novel host plant environment, I did not see more fixed alleles in coriacea-flies, potentially because the additional generations of glabra-flies allows selection to more efficiently remove slightly deleterious alleles. Regardless, the detection of divergent selection suggests host forms of *P. glabricola* are in the midst of ecological speciation.

Recent studies of speciation have often identified divergent selection in genomic areas of reduced recombination. I did not find evidence of linkage disequilibrim among outliers, as expected if outliers are within an inversion, nor evidence of outliers on sex chromosomes. I cannot, however, eliminate the possibility of LD or sex-linkage due to the low genomic resolution of AFLP loci in this study.

The endemic *P. glabricola* belongs to an adaptive radiation of leaf-mining flies onto *Ilex* species. Although not guaranteed, it is reasonable to presume the macroevolutionary patterns seen in *Phytomyza* are due to similar microevolutionary processes. Because *P. glabricola* is either currently diverging or recently diverged, it is an appropriate species with which to identify the evolutionary processes responsible for an adaptive radiation. It appears that ecological speciation may be that mechanism. Future work will need to determine how other host races and recently diverged species

have evolved within this clade of *Phytomyza*, which has great potential to become a model system for the evolution of new species. Future work should also focus on what trait(s) are under divergent selection, the genetic basis for these traits, and the resulting phenotypes to fully grasp the evolutionary mechanisms driving divergence in these flies.

Table 2.1. AFLP and EF-1α primer sequences. *Pst1*A was used in combination with each of the *Eco*RI based primers (*E*ACA-*E*AGT).

Primer	Sequence
AFLP	
*Pst*1A	5 ’- GAC TGC GTA CAT GCA GA - 3’
*E*ACA	5’ - /56-FAM/GAC TGC GTA CCA ATT CAC A - 3’
*E*ACT	5’ - /56-FAM/GAC TGC GTA CCA ATT CAC T - 3’
*E*AGA	5’ - /56-FAM/GAC TGC GTA CCA ATT CAG A - 3’
*E*AGT	5’ - /56-FAM/GAC TGC GTA CCA ATT CAG T - 3’
EF-1α	
EF46F *	5' - GAG GAA ATC AAG AAG GAA G - 3'
PEF40F	5' - TCG TCA TTG GAC ACG TAG ATT CAG G - 3'
PEF61R	5' - GAT GGT TCC AAC ATG TTA TCA C - 3'
PEF64R	5' - CGA CAC ATA AAG GCT TGG ATG GCA CC - 3'
PEF65R	5' - GTC TCA TGT CAC GCA CAG CGA AAC GAC - 3'

*(Cho et al. 1995)

Table 2.2. Summary of samples genotyped from each location and year.

			Coriacea-flies					Glabra-flies				
			Ef1alpha			AFLP		Ef1alpha			AFLP	
State	**Site**	**Population**	S&H[1]	2006	2007	2006	2007	S&H[1]	2006	2007	2006	2007
FL	Apalachicola National Forest	Hunters	-[2]	-	6	-	6	-	-	1	-	1
	Archibold Biological Station	Archibold	-	-	-	-	-	8	-	-	-	-
	Etoniah Creek State Forest	East V	-	-	-	-	-	-	-	2	-	0
		Stuck in Sand	-	-	7	-	5	-	-	11	-	9
GA	Crooked River State Park	Crooked River	-	-	-	-	-	-	-	4	-	3
SC	Francis Marion National Forest	Big Ocean Bay	10	17	5	15	4	-	18	6	17	4
		Wambaw Trail	-	19	10	12	7	-	21	1	16	2
NC	Croatan National Forest	Catfish Lake	-	22	-	18	-	-	5	-	3	-
		Road 152	-	22	10	20	7	-	25	4	19	2
	Carolina Beach State Park	Carolina Beach	15	-	-	-	-	7	-	-	-	-
VA	Great Dismal Swamp National Wildlife Refuge	Great Dismal Swamp	-	-	4	-	2	-	-	1	-	1
MD	Annapolis	Annapolis	-	-	-	-	-	2	-	-	-	-
DE	Cape Henlopen State Park	Cape Henlopen	-	-	-	-	-	-	-	15	-	10
NY	Long Island	Long Island	-	-	-	-	-	4	-	-	-	-
		Subtotal	25	80	42	63	31	21	69	45	55	32
		Total		147		96			135		87	

[1]Details on samples can be found in Scheffer and Hawthorne (2007). [2] Samples not collected from locations with '-'.

Table 2.3. Results from CVHAPLOT. Analyzing flies from each host plant separately yielded a better consensus between the programs.

CV category	H	S	I	II	III	Overall
Individuals (combined data)	127	139	13	9	8	296
Individuals (from *I. coriacea*)	90	53	2	2	1	148
Individuals (from *I. glabra*)	37	86	11	7	7	148
Number distinct genotypes	10	56	10	9	8	93
Total distinct haplotypes	10	33	14	16	14	57
Number of category-unique haplotypes*	10	25	8	7	7	57
Frequency (%) of category-unique haplotypes in total sample	83.9	11.5	2.2	1.2	1.2	100

Note: All rows following the separate host plant analyses refer to the combined data from those separate analyses. H: homozygous individuals; S: individuals where all programs fully supported the same haplotype; I – III: number of dissenting consensus votes received in each category (e.g., I means only one program had a different solution than the others); * Haplotypes newly observed in each category.

Table 2.4. Summary statistics for EF-1α sequence data.

	N	H	p	Sn	H_d	Π	Rm
Flies from *I. coriacea*	145	15	10	1	0.4932	0.047474	4
Flies from *I. glabra*	134	36	22	13	0.7869	0.104536	5
Total flies	279	43	22	12	0.8008	0.114040	6

N: number of phased samples; H: the number of haplotypes; p: the total number of polymorphic SNPs; Sn: the number of singleton haplotypes; H_d: haplotype diversity; π: nucleotide diversity; Rm: the minimum number of recombination events.

Table 2.5. Analysis of molecular variance estimated using the ADONIS function for AFLP data from *Phytomyza glabricola* feeding on either *Ilex coriacea* or *I. glabra*. Variation was partitioned **(a)** among individuals on each host plant species nested within each location, sex of the flies, and the collection year for North and South Carolina populations; **(b)** among individuals on each host nested within each location and sex of the flies; **(c & d)** among locations and sex of the flies within each host plant species. All non-significant interactions were removed from the analysis.

	Source	d.f.	SS	MS	F - model	R^2	P (>F)
a)	Location	1	0.33913	0.33913	2.38946	0.0126	**< 0.001**
	Sex	2	2.72593	1.36296	9.60317	0.1009	**< 0.001**
	Year	1	0.16667	0.16667	1.17435	0.0062	0.226
	Host nested in Location	2	4.05319	2.02659	14.27898	0.1500	**< 0.001**
	Residuals	139	19.72807	0.14193	- .	0.7303	- .
	Total	145	27.01300			1	
b)	Location	6	2.30711	0.38452	2.71925	0.0670	**< 0.001**
	Sex	2	3.23548	1.61774	11.44043	0.0939	**< 0.001**
	Host nested in Location	5	5.00615	1.00123	7.08055	0.1453	**< 0.001**
	Residuals	169	23.89756	0.14141	- .	0.6938	- .
	Total	182	34.44630			1	
c)	**Coriacea-flies**						
	Location	4	1.20764	0.30191	2.28281	0.0806	**< 0.0005**
	Sex	2	2.00981	1.00491	7.59829	0.1341	**< 0.0005**
	Residuals	89	11.77062	0.13225	- .	0.7853	- .
	Total	95	14.98807			1	
d)	**Glabra-flies**						
	Location	6	1.55257	0.25876	1.73281	0.1046	**< 0.0005**
	Sex	2	1.63939	0.81969	5.48911	0.1105	**< 0.0005**
	Residuals	78	11.64781	0.14933	- .	0.7849	- .
	Total	86	14.83977			1	

Table 2.6. Analysis of molecular variance estimated using the ADONIS function for EF-1α sequences from *Phytomyza glabricola* feeding on either *Ilex coriacea* or *I.glabra*. Variation was partitioned **(a)** among locations, year, and among individuals on each host plant nested within location for North and South Carolina populations (the only populations sampled in more than one year); **(b)** among locations and host plants nested within location; **(c & d)** among locations and sex of the flies within each host plant species. All non-significant interactions were removed from the analysis.

	Source	d.f.	SS	MS	F - model	R^2	P (>F)
a)	Location	1	-0.000003	-0.000003	- 3.9021	- 0.0046	1
	Year	1	0.000002	0.000002	2.2811	0.0027	0.1678
	Host nested in	2	0.000483	0.000242	314.17	0.7465	**< 0.0005**
	Location	215	0.000165	0.000001	- .	0.2554	- .
	Residuals	219	0.000647			1	
	Total						
b)		9	1.381623	0.153514	10.597825	0.1378	**< 0.0005**
	Location	5	4.823749	0.964750	66.601577	0.4810	**< 0.0005**
	Host nested in	264	3.824143	0.014485	- .	0.3813	- .
	Location	278	10.029514			1	
	Residuals						
c)	Total						
		4	0.000012	0.000003	6.8100	0.1639	**< 0.05**
	Coriacea-flies	139	0.000060	0.000000	- .	0.8361	- .
	Location	143	0.000072			1	
	Residuals						
d)	Total						
		9	0.18877	0.020974	13.744	0.4974	**< 0.05**
	Glabra-flies	125	0.19076	0.001526	- .	0.5026	- .
	Location	134	0.37953			1	
	Residuals						
	Total						

Table 2.7: Estimates of F_{ST} from AFLPs and EF-1α based on host plant (total samples), host plant within locations, and among locations within coriacea-flies and glabra-flies (separately). Samples from locations with less than five samples on one of the host plants were removed from all but the host plant comparison.

	AFLPs		EF-1α		AFLP outliers		AFLP non-outliers	
Comparison	F_{ST}	p-value	Φ_{ST}	p-value	F_{ST}	p-value	F_{ST}	p-value
Host plant	0.1247	< 0.0005	0.5166	< 0.0001	0.4946	< 0.0005	0.0571	< 0.0005
NC host plant	0.1270	< 0.0005	0.4950	< 0.0001	0.5045	< 0.0005	0.0632	< 0.0005
SC host plant	0.1390	< 0.0005	0.5599	< 0.0001	0.5179	< 0.0005	0.0764	< 0.0005
East-FL host plant	0.0973	0.0032	0.5892	< 0.0001	0.4154	< 0.0005	0.0553	0.0142
Locations of coriacea-flies	0.0482	0.0182	0.0284	0.0088	0.0883	0.0076	0.0312	0.0014
Locations of glabra-flies	0.0178	0.0146	0.0374	0.0968	0.0527	0.0834	0.0158	0.0322

Table 2.8. Summary statistics for AFLPs: **a)** all loci combined, **b)** outlier loci only, **c)** non-outlier loci only.

	Pop	n	#loc.	#poly. loc.	H_J	H_S
a)	Flies from *I. coriacea*	96	265	238	0.1559	0.1723
	Flies from *I. glabra*	87	265	232	0.1594	0.1771
	Total	183	265	265	0.1662	0.1577
b)	Flies from *I. coriacea*	96	15	12	0.2429	0.2404
	Flies from *I. glabra*	87	15	14	0.2594	0.2444
	Total	183	15	15	0.3430	0.2512
c)	Flies from *I. coriacea*	96	250	226	0.1508	0.1590
	Flies from *I. glabra*	87	250	218	0.1535	0.1646
	Total	183	250	250	0.1556	0.1521

n: number of samples; #loc.: number of loci; #poly loci.: number of polymorphic loci; H_J: Nei's gene diversity; H_S: average gene diversity within populations.

Table 2.9. Outliers detected using DFDIST from comparisons between all study populations. Dashes indicate the trimmed mean F_{ST} was too low a value to run DFDIST. 'Repeated across comparisons indicates' the number of loci with an outlier above 95% in more than one location comparison (number in independent comparisons).

			Outlier loci: 95% (99%)	
	Geographic Distance (km)	No. of polymorphic loci	Total	%
Across hosts				
C_{NC} vs. G_{DE}	430	187	11 (5)	5.9% (2.7%)
C_{NC} vs. G_{NC}	0	198	12 (8)	6.1% (4.0%)
C_{NC} vs. G_{SC}	312	214	13 (8)	6.1% (3.7%)
C_{NC} vs. $G_{E\text{-}FL}$	722	181	8 (2)	4.4% (1.1%)
C_{SC} vs. G_{DE}	752	177	10 (5)	5.6% (2.8%)
C_{SC} vs. G_{NC}	312	190	10 (8)	5.3% (4.2%)
C_{SC} vs. G_{SC}	0	215	13 (10)	6.0% (4.7%)
C_{SC} vs. $G_{E\text{-}FL}$	424	172	9 (3)	5.2% (1.7%)
$C_{E\text{-}FL}$ vs. G_{DE}	1175	115	3 (0)	2.6% (0%)
$C_{E\text{-}FL}$ vs. G_{NC}	722	143	5 (0)	3.5% (0%)
$C_{E\text{-}FL}$ vs. G_{SC}	424	170	14 (3)	8.2% (1.8%)
$C_{E\text{-}FL}$ vs. $G_{E\text{-}FL}$	0	104	6 (0)	5.8% (0%)
$C_{W\text{-}FL}$ vs. G_{DE}	1284	125	4 (0)	3.2% (0%)
$C_{W\text{-}FL}$ vs. G_{NC}	870	198	10 (4)	5.1% (2.0%)
$C_{W\text{-}FL}$ vs. G_{SC}	558	175	13 (7)	7.4% (4.0%)
$C_{W\text{-}FL}$ vs. $G_{E\text{-}FL}$	264	115	4 (3)	3.5% (2.6%)
Combined	na	257	15 (11)	5.7% (4.2%)
	Repeated across comparisons		23 (14)	8.7% (5.3%)
Within *I. coriacea*				
C_{NC} vs. C_{SC}	312	190	--	--
C_{NC} vs. $C_{E\text{-}FL}$	722	163	8 (5)	4.9% (3.1%)
C_{NC} vs. $C_{W\text{-}FL}$	870	165	7 (2)	4.2% (1.2%)
C_{SC} vs. $C_{E\text{-}FL}$	424	154	6 (3)	3.9% (1.9%)
C_{SC} vs. $C_{W\text{-}FL}$	558	155	6 (1)	3.9% (0.6%)
$C_{E\text{-}FL}$ vs. $C_{W\text{-}FL}$	264	78	5 (1)	6.4% (1.3%)
Combined	na	203	13 (7)	6.4% (3.4%)
	Repeated across comparisons		11 (0)	5.4% (0.0%)
Within *I. glabra*				
G_{DE} vs. G_{NC}	430	148	--	--
G_{DE} vs. G_{SC}	752	173	5 (2)	2.9% (1.1%)
G_{DE} vs. $G_{E\text{-}FL}$	1175	131	2 (1)	1.5% (0.8%)
G_{NC} vs. G_{SC}	312	185	--	--
G_{NC} vs. $G_{E\text{-}FL}$	722	192	3 (1)	1.6% (0.5%)
G_{SC} vs. $G_{E\text{-}FL}$	424	174	8(4)	4.6% (2.3%)
Combined	na	197	10 (4)	5.1% (2.0%)
	Repeated across comparisons		4 (0)	2.0% (0.0%)

Table 2.10. Summary of outlier loci found in host, sex, and geographic comparisons. Posterior probabilities in bold indicate marker found as an outlier in multiple independent population comparisons. Dashes indicate non-significant posterior probabilities (using an alpha of 0.05).

	Between hosts		**Within *I. coriacea***		**Within *I. glabra***		**Between sexes**	
Outlier # (name)	DFDIST	BAYESCAN	DFDIST	BAYESCAN	DFDIST	BAYESCAN	DFDIST	BAYESCAN
2 (eact.140)	--	--	--	--	--	--	**1**	1
8 (eact.210)	--	--	1	--	--	--	--	--
13 (eact.254.6)	**0.99975**	1	--	--	--	--	--	--
20 (eact.333.8)	--	--	--	--	--	--	**1**	1
22 (eact.349.5)	--	--	--	--	--	--	0.977256	--
28 (eact.392)	--	--	--	--	0.990752	--	--	--
32 (eact.407.4)	--	--	--	--	--	--	**1**	1
41 (eact.457.7)	--	--	--	--	--	--	**1**	1
43 (eact.472.2)	--	--	--	--	--	--	0.979505	--
51 (eact.537)	--	--	0.979505	--	--	--	--	--
70 (eaca.208.1)	**1**	1	0.990502	--	--	--	--	--
72 (eaca.219.3)	**0.99975**	1	0.99975	--	--	--	--	--
74 (eaca.253.3)	--	--	--	--	0.990502	--	--	--
92 (eaca.371.9)	--	--	1	--	--	--	--	--
94 (eaca.388.4)	**1**	1	--	--	--	--	--	--
99 (eaca.404.8)	--	--	--	--	--	--	**1**	--
109 (eaca.469.9)	--	--	--	--	0.986	--	--	--
111 (eaca.489.2)	--	--	0.996751	--	--	--	--	--
113 (eaca.505.8)	--	--	--	--	--	--	0.9915	--
115 (eaca.518.8)	**1**	1	--	--	--	--	--	--
116 (eaca.522.7)	--	--	0.997751	--	--	--	--	--
118 (eaca.532.1)	**1**	1	--	--	--	--	--	--
122 (eaca.584.8)	--	--	0.976256	--	--	--	--	--
124 (eaca.592.8)	--	--	--	--	--	--	**0.99925**	--
125 (eaca.623.9)	--	--	--	--	--	--	**1**	1
132 (eaca.755.4)	--	--	--	--	--	--	**1**	1
137 (eagt.148)	--	--	--	--	--	--	**0.99975**	0.997
144 (eagt.226.3)	--	--	--	0.952	--	--	--	--
148 (eagt.236.3)	--	--	0.984004	--	--	--	--	--
167 (eagt.414.1)	--	--	0.994251	--	0.997001	--	--	--
184 (eagt.552.8)	--	--	--	--	0.995001	--	--	--
188 (eagt.654.5)	--	--	--	--	--	--	0.991252	--
191 (eagt.729.2)	--	--	--	--	--	--	0.990002	--

	Between hosts		Within *I. coriacea*		Within *I. glabra*		Between sexes	
Outlier # (name)	DFDIST	BAYESCAN	DFDIST	BAYESCAN	DFDIST	BAYESCAN	DFDIST	BAYESCAN
192 (eagt.737.6)	--	--	--	--	--	--	**1**	1
193 (eagt.739.9)	--	--	--	--	0.993252	--	**1**	0.978
199 (eaga.186.1)	--	--	--	--	--	--	0.993252	--
200 (eaga.210.2)	**0.983754**	--	--	--	--	--	--	--
204 (eaga.249.1)	**0.998**	0.961	--	--	--	--	--	--
213 (eaga.297.7)	**0.994251**	0.989	--	--	--	--	--	--
225 (eaga.402.1)	--	--	0.995001	--	--	--	--	--
226 (eaga.411.3)	--	--	0.99925	--	0.997251	--	--	--
227 (eaga.425.2)	**0.99925**	0.992	--	--	--	--	--	--
229 (eaga.432.4)	--	--	--	--	0.9995	--	--	--
231 (eaga.437.4)	0.993252	0.956	--	--	--	--	--	--
238 (eaga.489.5)	**0.976006**	--	--	--	--	--	--	--
241 (eaga.498.2)	--	--	--	--	0.988503	--	--	--
242 (eaga.499.4)	**0.999251**	0.982	--	--	--	--	--	--
245 (eaga.517.6)	--	--	0.994501	--	--	--	--	--
246 (eaga.518.5)	**0.99925**	1	--	--	--	--	--	--
249 (eaga.542.9)	--	--	--	--	--	--	**1**	1
250 (eaga.543.9)	--	--	--	--	--	--	1	0.999
251 (eaga.583.9)	--	--	--	--	--	--	**1**	1
255 (eaga.651.2)	**1**	1	--	--	--	--	--	--
259 (eaga.672.3)	--	--	--	--	0.993252	--	--	--
260 (eaga.681.6)	--	--	--	--	--	--	**1**	--
261 (eaga.684.6)	--	--	--	--	--	--	**1**	1

Table 2.11. Distribution of peaks in host-associated outliers. Numbers represent the number of individuals that have a peak at that locus.

Locus	13	70	72	94	115	118	200	204	213	227	231	238	242	246	255	Total
Coriacea-flies																
VA	1	0	2	0	2	2	0	0	1	0	1	1	0	2	0	2
NC	39	0	41	7	13	44	23	0	38	0	26	26	1	16	0	45
SC	34	0	36	4	4	38	11	0	30	0	20	26	0	12	0	38
East-FL	4	2	0	0	2	4	0	0	5	0	2	0	1	2	0	5
West-FL	4	0	5	0	2	6	3	0	6	0	0	4	0	2	0	6
Frequency	0.85	0.02	0.88	0.11	0.24	0.98	0.39	0.00	0.83	0.00	0.41	0.59	0.02	0.35	0.00	
Glabra-flies																
DE	3	10	0	10	10	0	0	7	1	2	1	0	7	10	5	10
VA	0	1	0	0	1	0	0	1	0	0	0	0	1	1	1	1
NC	1	20	3	16	24	1	0	11	5	11	1	2	17	24	19	24
SC	2	27	2	36	36	1	0	13	10	23	0	4	18	36	34	39
GA	1	2	0	3	3	0	0	1	2	2	0	2	2	3	1	3
East-FL	1	6	3	8	9	0	0	1	1	4	0	2	2	9	9	9
West-FL	0	1	0	1	1	0	0	0	0	0	0	1	0	1	1	1
Frequency	0.09	0.77	0.09	0.85	0.97	0.02	0.00	0.39	0.22	0.48	0.02	0.13	0.54	0.97	0.80	

Frequency: the frequency of peaks within the listed host form (coriacea-flies or glabra-flies).

Table 2.12. Estimates of allele frequencies for host-associated outlier loci treating males and females of each host race separately. Male frequencies were estimated treating males as haploids and as diploids to compare to estimates using female loci. If haploid male estimates are more similar to female estimates than diploid male estimates (see Table 2.13), the locus will be treated as putatively on the X-chromosome. If females have no peaks present (all 0 alleles) and males have peaks, the locus is putatively on the Y-chromosome.

		Coriacea-flies						**Glabra-flies**					
outliers		haploid male		female		diploid male		haploid male		female		diploid male	
#	Locus	Freq.	SE	Freq.	SE	Freq.	SE	Freq.	SE	Freq.	SE	Freq.	SE
13	eact.254.6	0.8696	0.0025	0.5959	0.0049	0.6388	0.005	0.0952	0.0021	0.0488	0.0011	0.0488	0.0011
70	eaca.208.1	0.0217	0.0005	0.0103	0.0002	0.0109	0.0002	0.9048	0.0021	0.3828	0.0056	0.6914	0.0051
72	eaca.219.3	0.8193	0.0021	0.622	0.0048	0.6703	0.0048	0.0714	0.0016	0.0614	0.0014	0.0364	0.0008
94	eaca.388.4	0.1522	0.0028	0.0417	0.0008	0.0792	0.0016	0.881	0.0025	0.5636	0.0059	0.655	0.0054
115	eaca.518.8	0.3696	0.0051	0.0632	0.0012	0.206	0.0036	1	0	0.7327	0.0047	1	0
118	eaca.532.1	0.9783	0.0005	0.8571	0.0025	0.8526	0.0027	0	0	0.012	0.0003	0	0
200	eaga.210.2	0.413	0.0053	0.1919	0.0032	0.2339	0.0039	0	0	0	0	0	0
204	eaga.249.1	0	0	0	0	0	0	0.3571	0.0055	0.2441	0.0044	0.1982	0.0038
213	eaga.297.7	0.8696	0.0025	0.5482	0.0051	0.6388	0.005	0.1429	0.0029	0.1409	0.0029	0.2763	0.0048
227	eaga.425.2	0	0	0	0	0	0	0.4762	0.0059	0.2763	0.0048	0.2763	0.0048
231	eaga.437.4	0.5	0.0054	0.3149	0.0044	0.2929	0.0045	0.0238	0.0006	0	0	0.012	0.0003
238	eaga.489.5	0.3696	0.0051	0.5482	0.0051	0.206	0.0036	0.0952	0.0021	0.0742	0.0016	0.0488	0.0011
242	eaga.499.4	0.0217	0.0005	0.0103	0.0002	0.0109	0.0002	0.5714	0.0058	0.3274	0.0052	0.3453	0.0054
246	eaga.518.5	0.4348	0.0053	0.1548	0.0027	0.2482	0.0041	1	0	0.7327	0.0047	1	0
255	eaga.651.2	0	0	0	0	0	0	0.7857	0.004	0.5636	0.0059	0.5371	0.0059

Freq.: estimated allele frequency. SE: standard error of allele frequency estimate.

Table 2.13. T-tests comparing estimated allele frequencies from Table 2.12. Comparisons were made between haploid male frequencies and female frequencies, then between diploid frequencies and female frequencies. Significantly different comparisons are primarily between haploid male estimated frequencies and female frequencies. The remaining significant differences between diploid male estimates and female estimates are also significantly different for haploid estimates as well, with the exception of locus 238.

		Coriacea-flies						**Glabra-flies**					
Outliers		**haploid male vs. female**			**diploid male vs. female**			**haploid male vs. female**			**diploid male vs. female**		
#	locus	t	s	p-value	t	s	p-value	t	s	p-value	t	s	p-value
13	eact.254.6	22.0305	0.0124	**0.0007** *	2.9696	0.0144	**0.0324**	5.3158	0.0087	**0.0109**	0	0.0072	0.3183
70	eaca.208.1	2.9483	0.0039	**0.0328**	0.2067	0.0029	0.3053	38.5523	0.0135	**0.0002** *	19.3343	0.0160	**0.0008** *
72	eaca.219.3	16.4639	0.0120	**0.0012** *	3.3958	0.0142	**0.0254**	1.1832	0.0085	0.1326	3.4542	0.0072	**0.0246**
94	eaca.388.4	12.5766	0.0088	**0.0020**	5.2454	0.0071	**0.0112**	22.4436	0.0141	**0.0006** *	5.5723	0.0164	**0.0099**
115	eaca.518.8	26.3357	0.0116	**0.0005** *	14.0876	0.0101	**0.0016** *	25.2682	0.0106	**0.0005** *	25.2682	0.0106	**0.0005** *
118	eaca.532.1	15.4061	0.0079	**0.0013** *	0.4296	0.0105	0.2687	4.4900	0.0027	**0.0150**	4.4900	0.0027	**0.0150**
200	eaga.210.2	16.4559	0.0134	**0.0012** *	3.4283	0.0123	**0.0250**	0	0	0.3183	0	0	0.3183
204	eaga.249.1	0	0	0.3183	0	0	0.3183	7.3601	0.0154	**0.0058**	3.2850	0.0140	**0.0270**
213	eaga.297.7	25.5345	0.0126	**0.0005** *	6.2111	0.0146	**0.0080**	0.1702	0.0118	0.3093	10	0.0135	**0.0032**
227	eaga.425.2	0	0	0.3183	0	0	0.3183	12.5241	0.0160	**0.0020**	0	0.0151	0.3183
231	eaga.437.4	12.8595	0.0144	**0.0019**	1.6061	0.0137	0.0889	6.2969	0.0038	**0.0078**	4.4900	0.0027	**0.0150**
238	eaga.489.5	12.1818	0.0147	**0.0021**	25.3417	0.0135	**0.0005** *	2.2374	0.0094	0.0530	3.1679	0.0080	**0.0288**
242	eaga.499.4	2.9483	0.0039	**0.0328**	0.2067	0.0029	0.3053	15.0771	0.0162	**0.0014** *	1.1267	0.0159	0.1403
246	eaga.518.5	21.4549	0.0131	**0.0007** *	7.7771	0.0120	**0.0052**	25.2682	0.0106	**0.0005** *	25.2682	0.0106	**0.0005** *
255	eaga.651.2	0	0	0.3183	0	0	0.3183	14.4662	0.0154	**0.0015** *	1.5810	0.0168	0.0910

Values in bold font are significantly different at a standard 0.05 level
t: the estimated t-value; s: the combined standard deviation. * Significantly different after a Bonferroni correction for multiple comparisons.

Table 2.14. Allele frequency estimates of sex-associated outliers treating males and females of each host race separately. Male frequencies were estimated treating males as haploids and as diploids to compare estimates using female loci. If haploid male estimates are more similar to female estimates than diploid male estimates (see Supp. Table 10), the locus will be treated as putatively on the X-chromosome. If females have no peaks present (bolded values of all 0 alleles) and males have peaks, the locus is putatively on the Y-chromosome. Bolded outliers were found to be in linkage disequilibrium with host-associated outlier 238.

Table 2.14

		Coriacea-flies						Glabra-flies						
outliers		haploid male		female		diploid male		haploid male		Female		diploid male		
#	locus	Freq.	SE	Freq.	SE	Freq.	SE	Freq.	SE	Freq.	SE	Freq.	SE	Chromosome
2	eact.140	0.5435	0.0054	0.0206	0.0004	0.3243	0.0048	0.7143	0.0049	0.0364	0.0008	0.4655	0.0059	Y?
20	**eact.333.8**	0.9348	0.0013	0.0742	0.0014	0.7446	0.0041	1	0	0.0120	0.0003	1	0	Y?
22	eact.349.5	0.0435	0.0009	0.0103	0.0002	0.0220	0.0005	0.2143	0.0040	**0**	**0**	0.1136	0.0024	Y?
32	**eact.407.4**	0.5000	0.0054	0.0311	0.0006	0.2929	0.0045	0.5238	0.0059	0.0120	0.0003	0.3099	0.0051	Y?
41	**eact.457.7**	0.8043	0.0034	0.0103	0.0002	0.5577	0.0054	0.9286	0.0016	**0**	**0**	0.7327	0.0047	Y?
43	eact.472.2	0.7174	0.0044	0.7143	0.0042	0.4684	0.0054	0.9762	0.0006	1	0	0.8457	0.0031	X?
99	eaca.404.8	0.1739	0.0031	**0**	**0**	0.0911	0.0018	0.2143	0.0040	**0**	**0**	0.1136	0.0024	Y
113	eaca.505.8	0.6304	0.0051	0.7143	0.0042	0.3921	0.0052	0.6667	0.0053	0.5371	0.0059	0.4226	0.0058	X?
124	eaca.592.8	0	0	0.0853	0.0016	0	0	0.0476	0.0011	0.1691	0.0033	0.0241	0.0006	Auto?
125	**eaca.623.9**	0.9348	0.0013	**0**	**0**	0.7446	0.0041	0.9762	0.0006	**0**	**0**	0.8457	0.0031	Y
132	**eaca.755.4**	0.9348	0.0013	0.0103	0.0002	0.7446	0.0041	0.9524	0.0011	**0**	**0**	0.7818	0.0041	Y?
137	eagt.148	0.2609	0.0042	**0**	**0**	0.1403	0.0026	0.4286	0.0058	0.0120	0.0003	0.2441	0.0044	Y?
188	eagt.654.5	0.9783	0.0005	1	0	0.8526	0.0027	0.8571	0.0029	1	0	0.6220	0.0056	X?
191	eagt.729.2	0.1739	0.0031	**0**	**0**	0.0911	0.0018	0.1190	0.0025	0.0120	0.0003	0.0614	0.0014	Y?
192	eagt.737.6	0.7391	0.0042	1	0	0.4892	0.0054	0.7143	0.0049	1	0	0.4655	0.0059	X?
193	eagt.739.9	0.2174	0.0037	**0**	**0**	0.1153	0.0022	0.2381	0.0043	**0**	**0**	0.1271	0.0026	Y
199	eaga.186.1	0.1739	0.0031	**0**	**0**	0.0911	0.0018	0.1429	0.0029	0.0120	0.0003	0.0742	0.0016	Y?
249	**eaga.542.9**	0.9565	0.0009	**0**	**0**	0.7915	0.0036	0.9048	0.0021	**0**	**0**	0.6914	0.0051	Y
250	eaga.543.9	0.0217	0.0005	0.4467	0.0050	0.0109	0.0002	0.0238	0.0006	0.0614	0.0014	0.0120	0.0003	Auto?
251	**eaga.583.9**	0.9130	0.0017	0.0103	0.0002	0.7051	0.0045	0.8095	0.0037	**0**	**0**	0.5636	0.0059	Y?
260	eaga.681.6	0.0870	0.0017	0.1548	0.0027	0.0445	0.0009	0.0714	0.0016	0.2929	0.0049	0.0364	0.0008	X?
261	**eaga.684.6**	0.7391	0.0042	**0**	**0**	0.4892	0.0054	0.6429	0.0055	0.0120	0.0003	0.4024	0.0057	Y?

Figure 2.1: Endemic range of the host plants, *Ilex coriacea* and *I. glabra* with collection sites labeled.

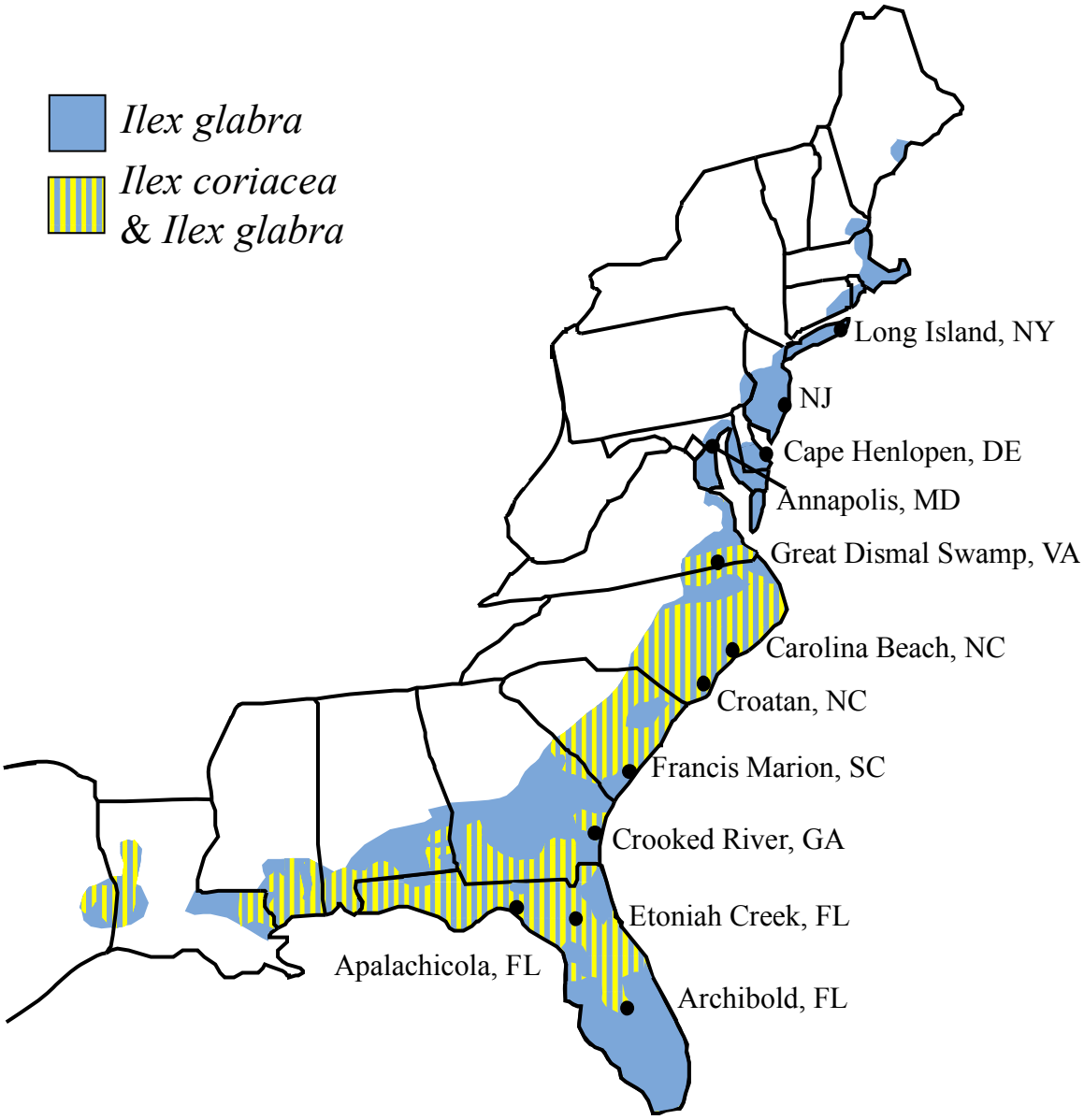


Figure 2.2. Alignment of translated EF-1α from *Phytomyza glabricola* to EF-1α-100e and EF-1α-48d from *Drosophila melanogaster*.

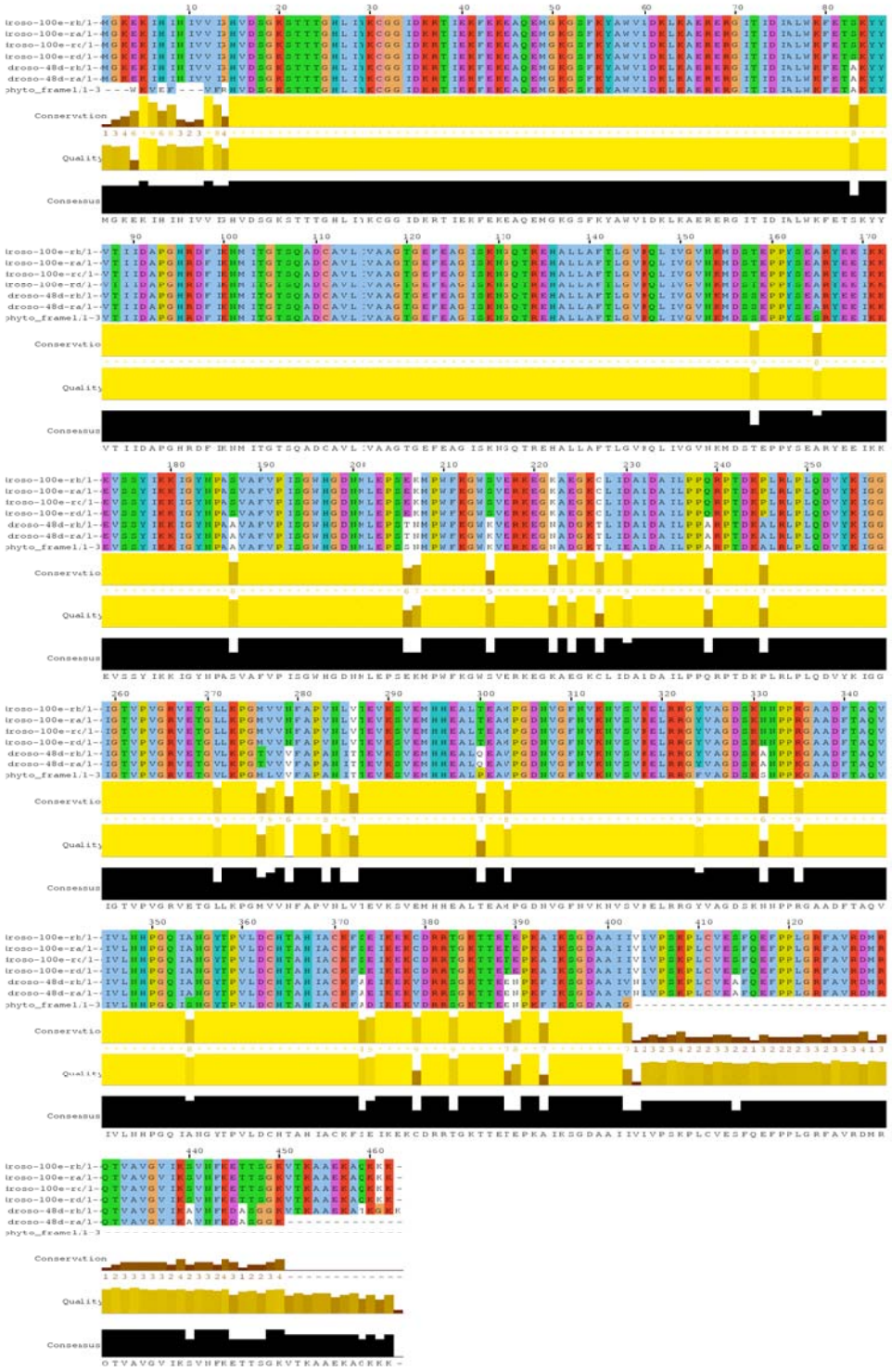


Figure 2.3: Spider diagrams of environmental factors fitted onto the ordination of AFLP data using non-metric multidimensional scaling. Lines connect each individual within a category to the centroid for that category. a) Host plant species; b) Sex of the fly.

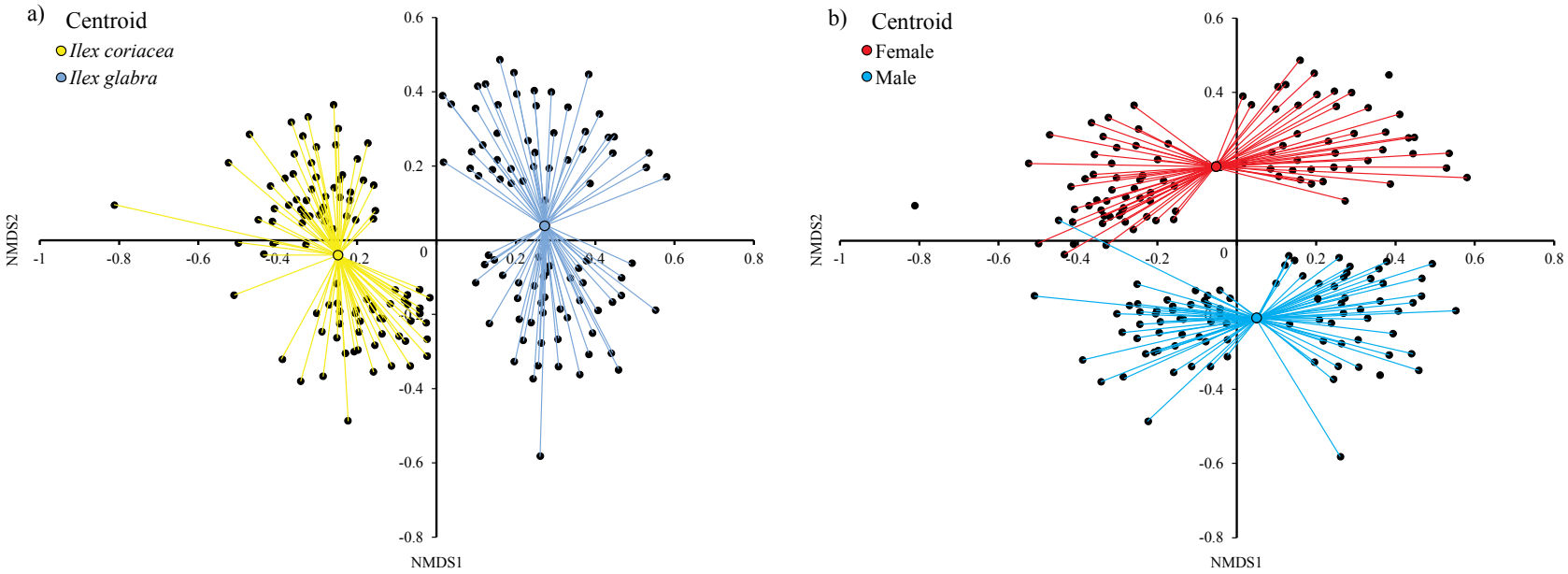


Figure 2.4. Results of non-metric multidimensional scaling (NMDS) of AFLPs. Yellow represents flies from *I. coriacea* and blue represent flies collected from *Ilex glabra.* Squares represent male flies and triangles are female flies. Four samples were genotyped as larvae, therefore their sex is unknown.

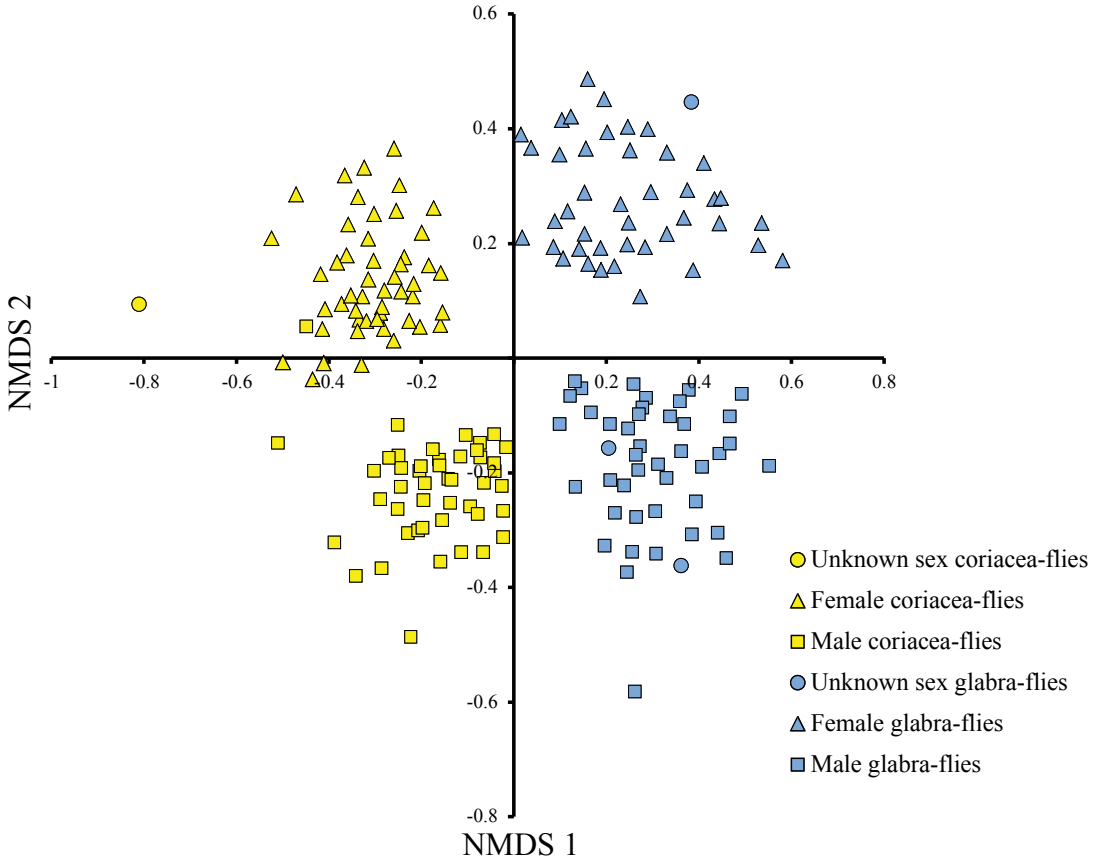


Figure 2.5. Haplotype network of EF-1α in *P. glabricola*. The size of nodes reflects the relative abundance of each haplotype in the total population. Nodes are colored based upon the frequency of flies from each host plant with that haplotype. Nodes are arranged to show size and connections, therefore connection length does not reflect the number of base pair changes between each haplotype. Each connection represents one base pair difference between nodes. The network is rooted by three closely related species: *P. ilicis, P. ditmani, and P. ilicicola.*

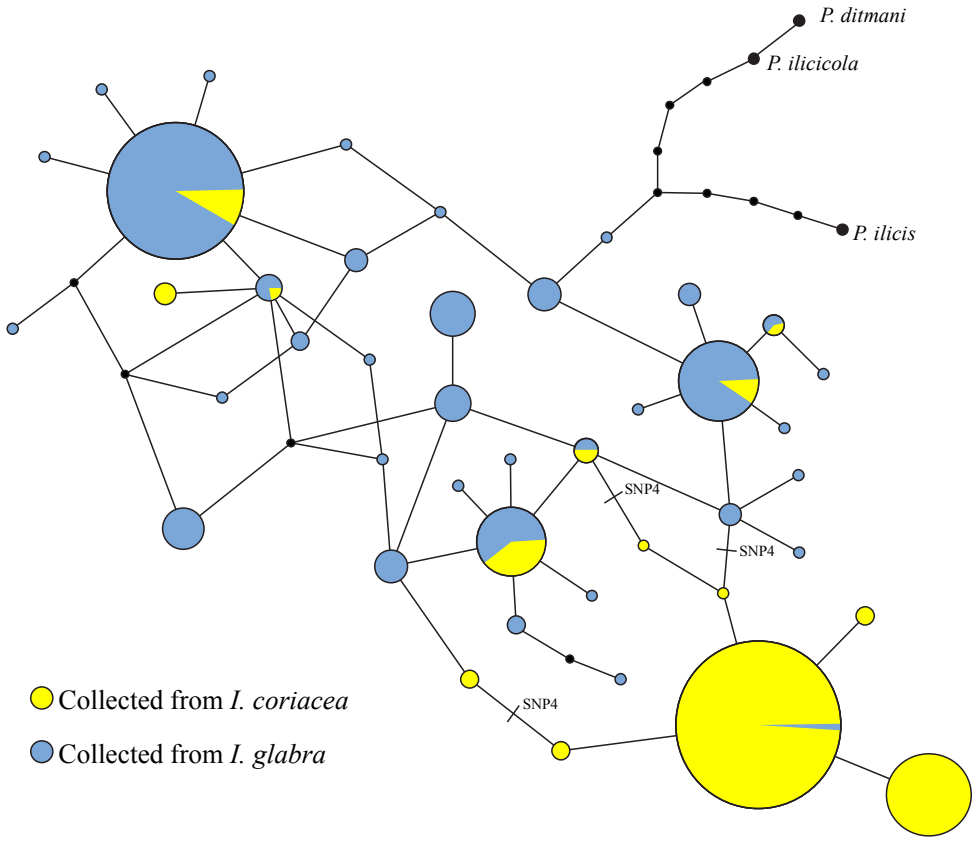


Figure 2.6: Haplotype network of EF-1α in *P. glabricola*. The size of nodes reflects the relative abundance of each haplotype in the total population. Nodes are colored based upon the frequency of flies from each location with that haplotype. Nodes are arranged to show size and connections, therefore connection length does not reflect the number of base pair changes between each haplotype. Each connection represents one base pair difference between nodes. The network is rooted by three closely related species: *P. ilicis, P. ditmani, and P. ilicicola.*

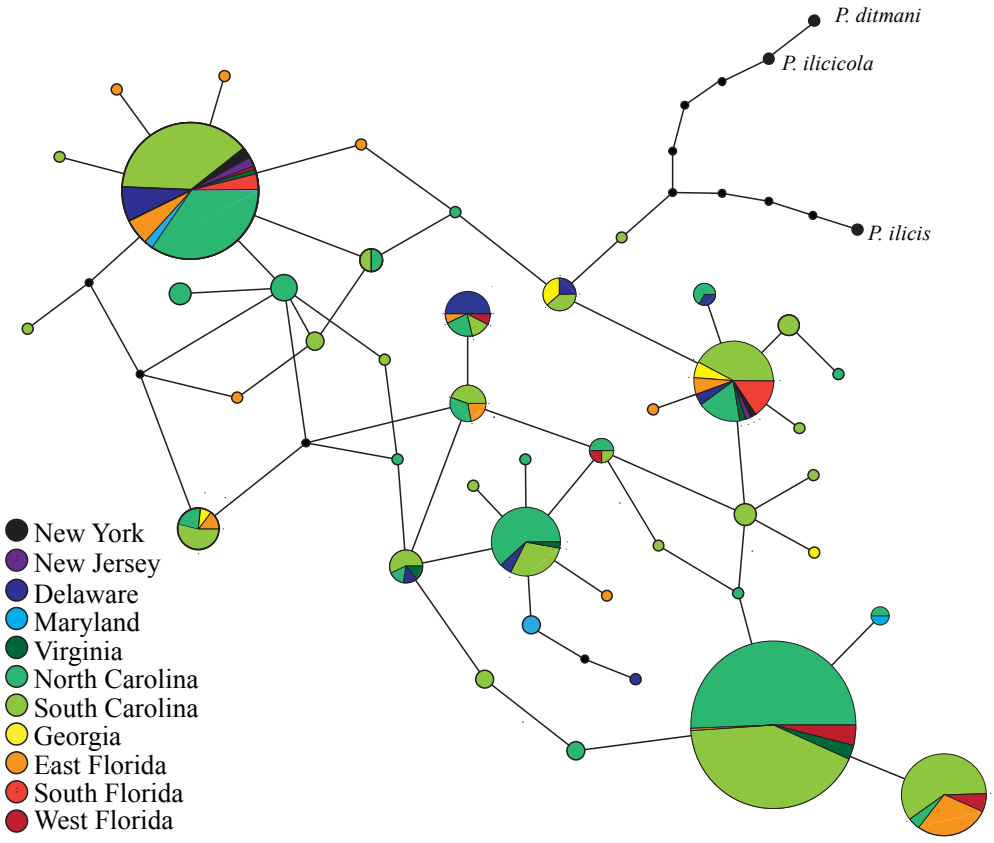
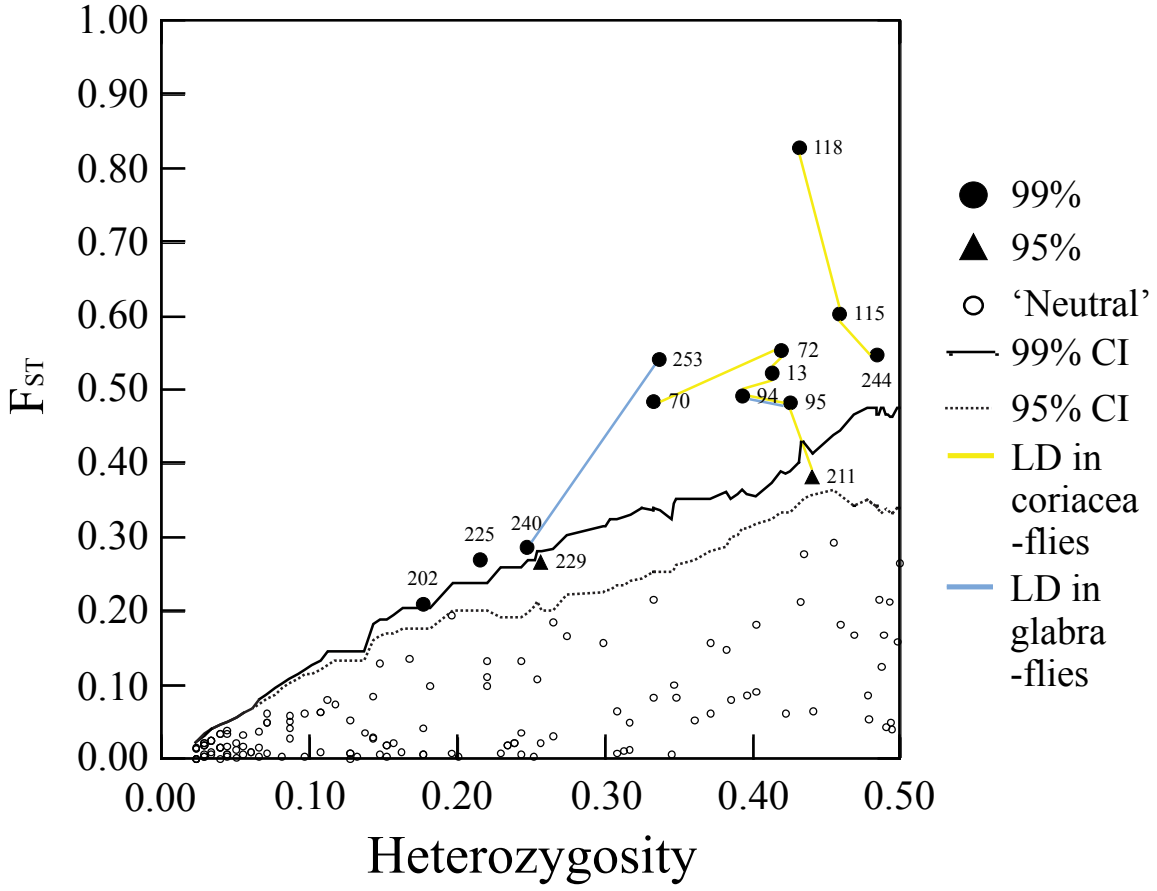


Figure 2.7. Results from among host plant comparison in DFDIST. Lines represent the 95% and 99% confidence intervals generated from the trimmed mean F_{ST} in DFDIST.



Note: Loci connected in yellow were in LD within coriacea-flies. Loci connected in blue were in LD within glabra-flies

CHAPTER 3: A GEOGRAPHIC MOSAIC OF HYBRIDIZATION BETWEEN *ILEX CORIACEA* AND *I. GLABRA* (AQUIFOLIACEAE) AND ITS EFFECTS ON HYBRID MORPHOLOGY

ABSTRACT

Premise: Interspecific hybridization is common in plants and can cause discordance among phylogenies based on different genes or phenotypes, particularly in taxa with porous genomes such as the genus *Ilex*. In these taxa, it is important to be able to identify and remove hybrid individuals from phylogenetic studies. I use a pair of sister species to test whether morphological characters can be used to reliably identify parental species and their hybrids.

Methods: Leaves were sampled from locations across the sympatric range of *I. coriacea* and *I. glabra*. AFLPs were used to genetically identify parental species and their hybrids. Discriminant functions were generated based on morphological characters of leaves to determine whether leaf morphology could reliably recover genetic identities.

Key Results: Natural hybrids were found in 3 of the 7 populations sampled, with asymmetric bidirectional gene flow of *I. glabra* alleles into the *I. coriacea* genetic background. Discriminant functions based on morphological characters were able to correctly identify all samples, but only if samples were first split into geographic regions, likely reflecting varying rates of hybridization among locations. No single trait could easily differentiate hybrids from parental samples, and each hybrid had a combination of parental, intermediate, and/or transgressive traits.

Conclusions: A geographic mosaic of hybridization exists across the range of *I. coriacea* and *I. glabra* resulting in a phenotypic mosaic of parental, intermediate, and transgressive

traits. The effects of hybridization in *Ilex* will likely depend on the individuals sampled, the location they are sampled from, and the traits examined.

INTRODUCTION

Hybridization is a common phenomenon in plants, particularly in outcrossing species with vegetative reproduction (Ellstrand et al. 1996; Rieseberg 1997). Although interspecific hybrids typically constitute less than 0.1% of a given population of hybridizing species, 25% of plant species are known to hybridize with at least one other species (Mallet 2005). Hybrids experience increased genetic variation via new combinations of alleles (Rieseberg and Ellstrand 1993) which can have a variety of outcomes. These combinations are often deleterious, but in some cases they can contribute to adaptability by producing novel phenotypes (Rieseberg and Carney 1998; Rieseberg et al. 1999; Whitham et al. 1999; Rieseberg et al. 2000) and allowing adaptive traits to introgress into a novel genomic background (Morgan et al. 2010). In addition new genetic combinations in hybrids can either decrease or increase differentiation between parental species (Seehausen 2004; Mallet 2005) by either breaking down or reinforcing reproductive barriers (reviewed in Abbott 1992; Rieseberg and Wendel 1993). Hence, hybridization can affect both anagenesis and cladogenesis in plant lineages and given its prevalence across taxa, it likely has been an important influence in plant diversification patterns and processes

The effects of hybridization on phylogenetic analysis are likely to be greatest in taxa with porous genomes, i.e., taxa that remain distinct entities despite current gene flow (Lexer et al. 2009). Such taxa are expected to have a ‘genetic mosaic’ of highly divergent and introgressed genome regions, depending on physical proximity to loci directly or indirectly involved in reproductive isolation (Wu 2001; Smadja et al. 2008; Via and West 2008). Such genetic mosaics should also result in ‘phenotypic mosaics’ due to

interspecific recombination in hybrids and backcrosses (Lexer et al. 2009). These complexities explain why few general patterns have emerged from studies that have examined phenotypes in hybrid plants (reviewed in Rieseberg and Ellstrand 1993). First generation hybrids are no more likely to display morphologically intermediate characters than parental ones, and most hybrids show at least one transgressive (i.e., extreme) phenotype (Rieseberg and Ellstrand 1993; Rieseberg et al. 1999).

The variation in both genetic and morphological characters in hybrid samples can be problematic for taxonomic and phylogenetic analysis. Intermediate morphologies can make species characterization difficult, and transgressive phenotypes can lead to long-branch attraction and homoplasy (Kornet and Turner 1999; Vriesendorp and Bakker 2005). Phylogenetic studies can exclude putative hybrids to gain phylogenetic clarity, but to do so, the hybrid individuals must be identified, and most phylogenetic studies do not include more than two or three individuals per species, making identification of hybrids difficult, especially in taxa with porous genomes where hybrids can form between both closely and distantly related taxa (Lexer et al. 2009; Manen et al. 2010). In these systems, more work is needed using multiple specimens of putatively hybridizing species to screen for hybrid individuals and phenotypes and determine whether genetic or morphological data will be more reliable for determining taxonomic relationships.

The family Aquifoliaceae (hollies) has emerged as a group with porous genomes. Recent work has revealed high levels of introgression between both closely and distantly related species and a lack of concordance between phylogenies based on different genes or morphological characters (Baas 1978; Cuenoud et al. 2000; Manen et al. 2002; Manen 2004; Selbach-Schnadelbach et al. 2009; Manen et al. 2010). Aquifoliaceae consist of a

single extant genus (Powell et al. 2000), *Ilex* (L.), of approximately 600 species (Loizeau et al. 2005). Taxonomic studies of *Ilex* noted the overlap in morphological variation among species, suggesting hybridization was likely an important part of evolution in the lineage (Baas 1978). Detailed population level studies of *Ilex* have identified naturally occurring hybrid individuals (Manen 2004; Lee et al. 2006) and hybrid species (Setoguchi and Watanabe 2000; Joung et al. 2011), indicating hybridization is a common phenomenon in *Ilex*. To date however, work has focused primarily on documenting the presence of hybrids rather than examining rates of hybridization or characterizing the genetic and morphological traits exhibited.

One pair of species, *I. coriacea* (Pursh) Chapm. and *I. glabra* (L.) A. Gray are consistently placed as sister taxa (but see Selbach-Schnadelbach et al. 2009) although the placement of this pair relative to other *Ilex* species varies between plastid and nuclear phylogenies (Manen et al. 2010). These species are evergreen holly shrubs that are native to pine forests on the coastal plain of the eastern United States (Duncan and Duncan 1987; Godfrey 1988). The more cold-tolerant *I. glabra* grows from Nova Scotia, south to Florida, and along the Gulf of Mexico into eastern Texas whereas *I. coriacea* has a much smaller range from southern Virginia south to northern Florida and west to Texas (Scheffer 2002; Chapters 1, 2). Throughout the range of *I. coriacea, I. glabra* is more abundant (Mohlenbrock 1976; Richardson 1983; Brewer 1998; Brockway and Lewis 2003; Clark et al. 2008), but where *I. coriacea* is found, the two species are sympatric and often syntopic. They are distinguished in the field based on leaf morphology (Gray and Fernald 1950; Lundell 1961; Duncan and Duncan 1987; Godfrey 1988; Lance 2004) but are often mistaken as a single species, particularly in southern populations (Lundell

1961). Due to the spatial overlap, the two species have potential to hybridize throughout the overlapping range, and plants with intermediate leaf morphology have been found throughout regions of overlap (Robert K. Godfrey Herbarium 2012, Specimens 000016759-000016766). However the hybrid status of these individuals has not been genetically confirmed.

The sympatric distribution, sister species status, and morphological similarities of *I. coriacea* and *I. glabra* are particularly suited to test whether morphological characters can be used to reliably identify parental species and their hybrids. For the purposes of this study, I use 'hybrid' to encompassing F1 and backcrossed individuals (i.e., non-parental types). Both *I. coriacea* and *I. glabra* are evergreen species and leaves are used to identify and differentiate them year round (Gray and Fernald 1950; Lundell 1961; Duncan and Duncan 1987; Godfrey 1988; Lance 2004). Hence, leaves were collected from multiple populations throughout the range of both species to encompass the full range of genotypic and morphological variation. The objectives of the study were to genetically confirm that *I. coriacea* and *I. glabra* naturally hybridize in wild populations and to determine whether hybridization rates vary among locations. In addition, I test whether morphology is a good indicator of hybrid status, especially in phenotypically plastic species.

METHODS

Collections

Plant material for genetic analysis was collected in January and February of 2006 and 2007 from Croatan National Forest, NC and Francis Marion National Forest, SC (Table 3.1). In 2007, additional samples were collected from Cape Henlopen State Park,

DE, the Great Dismal Swamp National Wildlife Refuge, VA, Crooked River State Park, GA, Etoniah Creek State Forest, FL, and Apalachicola National Forest, FL (Figure 3.1, Table 3.1). *Ilex glabra* was found at every collection site, however *I. coriacea* was not found at two of the sites (DE and GA), the first of which is outside the known geographic range of *I. coriacea*.

Plant material collected in 2007 was also used for morphometric analysis. Collection protocols were developed to represent variation across individuals and locations. *Ilex* can grow via vegetative reproduction, so the shrubs were selected by moving through a patch and collecting from plants clearly separated by one another by at least a yard between main trunks. The stem closest to the base of the plant with at least five leaves and no new growth was removed from each plant and placed into a plastic bag labeled with site and plant species. After returning to the lab, leaves were removed from the stem and images of both the abaxial and adaxial surfaces of the leaves were recorded using a scanner (Canon CanoScan LiDE 55) at 400 dpi with a ruler included to allow scaling for morphological measurement (Figure 3.2). Immediately after scanning, leaves were placed in labeled envelopes and stored at -80˚C.

AFLPs

Phylogenetic relationships of *Ilex* species based on nuclear data more closely resemble morphological relationships than do those based on plastid data (Manen et al. 2010); thus I used genomic markers to genetically differentiate each species and their putative hybrids. A total of 202 plants (104 putative *I. coriacea* and 98 putative *I. glabra*) were genotyped using AFLPs. For each plant, 35 to 45 mg of leaf material was frozen using liquid nitrogen and ground to a fine powder using a sterilized mortar and pestle.

Total genomic DNA was extracted following the plant tissue mini protocol of the Qiagen DNeasy plant kit (Qiagen, Valencia, CA) with a minor adjustment: the lysis step was incubated overnight to increase yield. Following extraction, DNA concentrations were standardized to 12.5 ng/μL. AFLPs were generated using two-step amplification (Vos et al. 1995; Chapter 2). Preamplification and amplification followed procedures in Chapter 2 with only a change in the selective primers used (Table 3.2). PCR products were separated with an ABI 3730 DNA Analyzer (Applied Biosystems, Carlsbad, CA) using MapMarker X-Rhodamine (ROX) labeled 1000bp ladder (BioVentures, Murfreesboro, TN).

Electropherograms were scored using GENEMARKER (Soft Genetics, LLC, State College, PA). Fragments between 76 and 949 base pairs were first scored using the automated procedure and secondarily checked by eye. Samples were then examined to determine the fragment size where peak heights became too low to be reliably scored. Final maximum fragment sizes varied from 457 to 720bp across primer pair combinations. AFLPs are known to exhibit problems with repeatability, therefore six individuals were repeated across plates, and ten individuals within each plate to test for repeatability, resulting in 85-136 repeated samples (depending on the optimization needed in each primer-pair combination). In addition, negative controls (H_2O template) were run for every step of the process.

After scoring, a genotyping error rate was estimated as the ratio of electropherogram peak mismatches among replicated samples to the total number of replicated markers (Pompanon et al. 2005). Using a conservative approach, loci with peaks in the negative controls were removed from the analysis as were loci with peak

mismatches among repeated samples. Mismatches are not equally distributed among loci: some loci have only a single individual with a mismatch whereas others show mismatches in a large number of individuals. As a result, the percentage of loci removed due to mismatches is much higher than the overall genotyping error rate. Finally, because a significant negative correlation of fragment frequency and fragment size may be caused by excessive homoplasy, the correlation was estimated using AFLPSURV (Vekemans et al. 2002).

Genetic Analysis

Local rates of hybridization can differ depending on ecological conditions (Bleeker and Hurka 2001; Williams et al. 2001; Watano et al. 2004; Aldridge and Campbell 2009) so I tested for geographic differences in genetic structure across the range of *I. coriacea* and *I. glabra*. Genetic differentiation and diversity were estimated for each species using AFLPs. Nei's genetic diversity, total gene diversity, Nei's H_S, and Wright's F_{ST} were calculated using AFLPSURV (Vekemans et al. 2002), with 5000 permutations run to test significance for F_{ST}. Geographic variation in genetic divergence was addressed using pairwise F_{ST} as calculated using AFLPSURV and using an analysis of molecular variance, performed using a permutational MANOVA of Jaccard distances via the ADONIS function from the VEGAN package in R (Oksanen et al. 2010). ADONIS models were constructed to test the effects of species and collection site location on the genetic structure of plants from all locations. Models were run with species nested within location to examine whether hybridization rates differed among locations. Significance was based on 5000 permutations producing pseudo-F ratios.

To identify rates of hybridization between the *Ilex* species, I used both a clustering algorithm and an assignment test of the AFLP genotypes. First, I used STRUCTURE (v2.3.3, Pritchard et al. 2000; Falush et al. 2007) to determine the number (K) of genetic groupings the hollies formed. Runs were conducted for K = 1 to 15 to determine whether genetic structure was present among species and sampled locations. I used the population admixture model and independently replicated each run of a given K 10 times with a burn-in of 125 000 iterations followed by 10^6 iterations of Monte Carlo Markov Chain (MCMC) via grid computing on the Lattice Project at the University of Maryland (Bazinet et al. 2007; Bazinet and Cummings 2008; Myers et al. 2008). To identify the most likely value of K among my samples I used ΔK as described in Evanno et al. (2005). The mean of the permutated matrices among replicates was calculated using CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007), then visualized using DISTRUCT 1.1 (Rosenberg 2004).

Although the result of STRUCTURE presents a hypothesized mixture of K populations, STRUCTURE does not assign individuals as having parental types or showing introgression associated with either hybridization or backcrossing. Thus, hybrid AFLP genotypes (F1 and backcrosses) were identified using NEWHYBRIDS (Anderson and Thompson 2002; Anderson 2008). Briefly, NEWHYBRIDS uses Bayesian inference to estimate the posterior probability that individuals belong in user-specified categories (e.g., parental, backcross, F1) based on the proportion of loci expected to come from one of two species (Anderson and Thompson 2002), and has been modified to allow inference using dominant markers (Anderson 2008). The choice of prior had no effect on the overall likelihood of the results, so calculations were run without individual-specific

assumptions using a 'Jeffreys-like' prior for the mixing proportion and a uniform prior for allele frequency. Simulations were run with a burn-in period of 8×10^4 iterations followed by 1.5×10^6 sweeps for sampling from the posterior distribution. Individuals were classified as parental, F_1, backcross, or late backcross depending on the probability of membership in each category. Parental-types were defined as having at least a 90% probability of being a parental form (Vaha and Primmer 2006). Within the hybrids, the category with the highest probability was considered true (F_1, backcross to *I. coriacea*, or backcross to *I. glabra*). In cases where the individual had the highest probability of being a parental-type, but that probability was less than 90%, the individual was considered a later generation backcross.

To determine whether a genetic mosaic of divergence exists for these species, frequencies for the presence of alleles were calculated for each locus for parental-type *I. coriacea* and separately for *I. glabra*. The frequencies of each species were visually compared using a scatterplot to determine whether some loci were more divergent than others.

Morphometric Analysis

To investigate whether vegetative morphological features can reliably differentiate the species and hybrids, I used morphometric analysis to distinguish individuals genotypically classified as *I. coriacea*, *I. glabra*, and hybrids. A total of 54 *I. coriacea* and 62 *I. glabra* samples were both genotyped and used for morphometric analysis. For each plant, I selected the largest leaf with the least amount of damage with no obvious discernible differences in shape from other leaves from that plant. Once the

leaf was chosen, landmarks were placed and stored as x-y coordinates and traditional measurements were made using TPSDIG2 (Rohlf 2005; Figure 3, Tables 3-5).

Strictly speaking, landmarks are defined as points at specific anatomical structures and are considered homologous, whereas pseudolandmarks are defined by specifying their position on a structure relative to each other and other landmarks present (Dickinson et al. 1987; Kores et al. 1993), therefore I am using. Pseudolandmarks have been shown to accurately represent shape (Dickinson et al. 1987) and are appropriate for this study because I am not testing for allometric change through time. Landmarks were placed at the base of the petiole, where the blade joined the petiole, and at the apex of the blade (Table 3.3, Figure 3.3). The length between the base and apex of the blade was measured, and landmarks were placed at ¼, ½, and ¾ the length of the blade on the midvein of the leaf. Landmarks were also placed on the edges of the leaf at a 90° angle to the midvein at ¼, ½, and ¾ of the length (Table 3.3, Figure 3.3).

Traditional morphological measurements were chosen based on features often used to differentiate these two species (Tables 3.4-3.5). Leaf shape ranges from elliptic to oblong for *I. coriacea* and obovate to elliptic for *I. glabra* (Lundell 1961; Godfrey 1988). The apex is described as acute or obtuse for *I. coriacea* with an acute, sometimes rounded base versus an obtuse apex and acute base in *I. glabra* (Lundell 1961; Godfrey 1988). In *I. coriacea*, the leaves are typically spinescent-serrate above the middle often with spinose prickles along the entire margin of the leaf (Gray and Fernald 1950; Duncan and Duncan 1987; Lance 2004). On the other hand, *I. glabra* leaves are typically crenate or crenate-serrate above the middle of the margin (Gray and Fernald 1950; Duncan and Duncan 1987). In addition, the leaves of *I. coriacea* tend to be larger than those of

I. glabra, and have a greater width relative to the length of the leaf (Lundell 1961; Godfrey 1988; Lance 2004).

All of the following analyses were conducted using the statistical package R (v2.11.1, 2010). Individuals were grouped by the resulting classifications from NEWHYBRIDS. Because there were very few individuals identified as hybrids and NEWHYBRIDS has been shown to be more robust at identifying hybrids versus parental individuals than discriminating between hybrid categories (Anderson 2008), all individuals with less than 90% parental membership were pooled as 'hybrids' for the remaining analyses (Vaha and Primmer 2006).

For analysis of landmark data, I performed generalized Procrustes analysis (GPA) using the function PROCGPA in the SHAPES package (Dryden 2009). Differences between the mean shapes of *I. glabra* and *I. coriacea*, and between parentals and hybrids, were tested using 5000 permutations of the tangent coordinates generated from the GPA's of each group in the function TESTMEANSHAPES, also in the SHAPES package.

The traditional morphological data was used to classify individuals into parental *I. coriacea*, *I. glabra*, and their hybrids via a discriminant function analysis (DFA) using the function LDA in the package MASS (Venables and Ripley 2002). Discriminant functions were generated using all of the samples collected, then using subsamples based upon location. Hybrid samples were not present in every location, so nearby locations had to be combined to allow for discrimination among parental species and hybrids. Treating genetic classification as 'truth', the performance of each of the discriminant functions was assessed using the following measures:

1) *Efficiency*, the power to identify true genotypic status of individuals (sensu Vaha and Primmer 2006; Burgarella et al. 2009): the number of correctly identified individuals for a group divided by the actual number of individuals actually in that group.
2) *Accuracy* (sensu Yang et al. 2005; Vaha and Primmer 2006; Burgarella et al. 2009): the proportion of individuals correctly assigned to a group divided by the total number of individuals assigned to that group.
3) *Type I error*: the number of individuals wrongly identified as hybrids over the total number of actual purebreds in a sample.

Finally, I examined each morphological trait in individual hybrids to characterize them as parental, intermediate, or transgressive trait states to determine whether a genetic mosaic is indeed tied to a phenotypic mosaic of leaf morphology. Means and standard deviations of each character were calculated for *I. coriacea* and *I. glabra*. A t-test for comparing a single observation to the mean was used to determine whether the character state of each hybrid individual fell within the range of *I. coriacea*, *I. glabra*, or both parental species(Sokal and Rohlf 1981). Character states significantly outside the range of both species were considered transgressive characters (Cosse et al. 1995).

RESULTS

Genetic analysis

A total of 1034 markers were scored giving an initial error rate of 8.82%. Discarding markers with a single discrepancy between repeated samples resulted in a total of 679 markers. Finally, an initial allele frequency cutoff of 0.5% (corresponding to only a single individual containing the rarer allele) resulted in a significant correlation

between fragment size and allele frequency ($N = 631$, $r = 0.0789$, $p < 0.05$). As a result, the frequency cutoff was increased until the correlation was no longer significant (at 3%, $N = 427$, $r = 0.0871$, $p = 0.07220$) to reduce the risk of homoplasy. The size range of the 427 AFLP markers was 76-720 bp and 79% had a fragment size above 200 bp.

Overall, there is more genetic diversity in *I. glabra* than in *I. coriacea*, with a larger number of polymorphic loci and larger estimates of genetic and gene diversity (Table 3.6). The results of ADONIS (Table 3.7) and analyses using F_{ST} yielded similar results, so only F_{ST} is given here. Populations of both holly species show small but significant genetic structuring among locations (*I. coriacea*: $F_{ST} = 0.0518$, $p < 0.0005$; *I. glabra*: $F_{ST} = 0.0290$, $p < 0.0005$; Table 3.6). The degree of divergence between the holly species varied in magnitude among locations with higher values of F_{ST} in northern than southern populations (Figure 3.4). Within *I. coriacea*, pairwise estimates of F_{ST} among populations indicated significant differences among all population comparisons except VA with NC, and eastern and western FL (Table 3.8a). Populations of *I. glabra* were much more similar to one another with significant differences between NC and all but DE, and between VA and western FL (Table 3.8b). When species were combined, the only locations with both host plants that showed significant divergence were eastern FL from NC and SC (Table 3.8c).

Using the AFLP data, STRUCTURE clustered the 202 individuals from the seven locations into two distinct groups that corresponded to species identification (Figures 3.5-3.6). Using a 90% membership cutoff, 97 samples were identified as *I. coriacea*, 95 as *I. glabra*, and 10 as hybrids (Appendix E). Only one sample was found to be

misidentified, initially identified as being *I. glabra* but conclusively *I. coriacea* based on its genotype (Appendix E).

The results from NEWHYBRIDS also indicated low rates of gene flow between the holly species. The same 97 individuals were identified as *I. coriacea* parentals, 95 as *I. glabra*, and 10 as hybrids (Appendix E). Hybrid individuals were found in NC, SC, and western FL, but not the other four locations. Of the 10 hybrids, 2 were identified as F1, 4 as backcrossed to *I. coriacea*, 3 as late backcrosses to *I. coriacea*, and 1 as a late backcross to *I. glabra*. Although the exact identification of sample status by NEWHYBRIDS may not be correct (Anderson 2008), the combination of population membership resulting from STRUCTURE and the number of individuals identified as backcrosses to *I. coriacea* give good evidence that gene flow is bidirectional, but asymmetric with primarily *I. glabra* alleles introgressing into the *I. coriacea* background.

When examining the loci for a genetic mosaic of divergence, the majority of loci were present at relatively high or low frequencies in both species (Figure 3.7). Several loci were absent from one species but present, at varying frequencies, in the others, and roughly 10% were at a high frequency in one location but low in the other, with a difference in frequency of 0.75 or more. A single locus was fixed among species: all *I. glabra* had a peak whereas all *I. coriacea* did not.

Morphometric Analysis

Preliminary analysis suggested *I. coriacea* and *I. glabra* could be discriminated based on leaf shape, but hybrids could not. Shapes of leaves based on landmarks were significantly different between parental *Ilex* species (James T^2: 84.35, $p < 0.01$), but not between parental plants and hybrids (James T^2: 561.44, $p = 0.387123$), the latter likely

due to a low number of hybrid plants (7 compared to 109 parental; Figure 3.8).Visual inspection of the mean shapes indicated hybrid leaves were, on the whole, transgressive rather than intermediate relative to the parental species (Figure 3.8d).

Discriminant function analyses were run using the full dataset then with combinations of samples that are geographically near one another. Samples from NC, SC, and/or western FL had to be included in each dataset as they were the only locations with individuals identified as hybrids (Appendix E). Because no hybrids were identified in DE or VA, the samples from these locations were combined with those from NC. Pairwise comparisons of F_{ST} among *I. coriacea* populations were significantly different between VA and SC, and NC and SC (Table 3.8a), as were comparisons among *I. glabra* populations between NC and SC (Table 3.8b), therefore samples from SC were not combined with the DE-VA-NC group. Samples from eastern and western FL were combined due to proximity and genetic similarity (Table 3.8). The GA population was more similar to the population from eastern FL than to SC, so it was combined with the FL populations, resulting in two groups: SC, and GA-FL.

The discriminant function based on the total data set did not perform as well as the functions based on the subsets of samples, which had no misclassified samples (Table 3.9, Figure 3.9). In the total combined data, hybrids and *I. coriacea* were more likely to be incorrectly identified (2 of 48 samples and 2 of 7 samples, respectively) than *I. glabra*, with only 1 sample misidentified. Similar to the mean shapes based on landmark data, linear discriminant scores of hybrid plants more closely resemble those of *I. coriacea* than *I. glabra* (Figure 3.9), potentially explaining why *I. glabra* was more likely to be correctly identified.

Hybrid individuals had character states that ranged from parental to intermediate to transgressive depending on the individual and character in question. No quantitative variables were particularly indicative of hybrid status, and most were intermediate between parental distributions (Table 3.10). Three hybrid individuals showed transgressive characters: one was identified as a backcross to *I. coriacea* (PHUNC012), one as an F_1 (PHUNGE05), and one as somewhere between an F_1 and a backcross to *I. coriacea* (PSOPC005; Appendix E). The three individuals identified as late backcrosses to *I. coriacea* (P152C288, P152CE02, and PBOBC191) all had a combination of intermediate and *I. coriacea*-like traits. Interestingly, one of the individuals identified as an F1 was intermediate for all characters (PBOBCE05), but the other had the largest number of transgressive character states (PHUNGE05).

Apex shape and leaf margin were the two most discriminatory qualitative characters that appeared representative of species status (Table 3.11). The majority of *I. coriacea* (37 out of 48 individuals) had complex apices whereas all 61 *I. glabra* had convex apices. Hybrids were split, 2 with convex apices and 5 with complex. The leaf margin of 40 of the 48 *I. coriacea* plants had bristles with no crenation whereas 54 of the 61 *I. glabra* had crenation on the leaf margin lacking bristles (Table 3.11). Hybrids more closely resembled *I. coriacea* with 6 of the 7 individuals containing bristles but no crenation along their leaf margins. The remaining hybrid, with crenation on the leaf margin, was the F_1 individual that also had the majority of the transgressive quantitative traits.

DISCUSSION

The objectives of this study were to test for hybridization between two species of holly, *I. coriacea* and *I. glabra*, examine whether hybridization rates varied among locations, and determine whether or not leaf morphology could be used to differentiate parental species from one another and hybrid plants. I found low rates of hybridization between these two species that varied depending on the location examined. These differences were also reflected in the ability to discriminate between the morphology of leaves of *I. coriacea*, *I. glabra*, and their hybrids: discriminant functions based on data divided into geographic regions were better able to correctly identify samples than the function based on the entire dataset. Despite the correct classification, there were no characters that could be used alone to discriminate samples and few patterns emerged regarding the character state of hybrids relative to parental types.

Geographic mosaic of hybridization

The genetic data match observations of the abundance and distribution of the plant species. *Ilex glabra* is both more abundant within a given location and has a wider geographic range than *I. coriacea*, likely generating the higher genetic diversity seen in *I. glabra*. The differences in genetic structure among the species are likely due to the patchier distribution of *I. coriacea*, resulting in a higher F_{ST} among its populations than among populations of *I. glabra*. Within a given host species, there was a trend for more northern populations to be similar to one another, but different than southern populations, suggesting different environmental pressures among northern and southern areas of the species' ranges.

Genetic data indicated that *I. coriacea* and *I. glabra* are naturally hybridizing in native populations. The degree of hybridization varies among geographic locations, with a general trend towards greater gene flow with a decrease in latitude. The genetic data agrees with observational data, where it can be more difficult to identify plants in southern populations (JBH, S. J. Scheffer*, personal observation*). Work in other systems have also found rates of hybridization can vary depending on ecological conditions such as differences in climate (Williams et al. 2001), pollinators (Chase and Raven 1975), and types of vegetation (Watano et al. 2004).

A number of factors could explain the geographic mosaic of hybridization in this system. In southern populations, *I. coriacea* begins blooming weeks before *I. glabra* (Godfrey 1988), but the degree of overlap in blooming time for these species is unknown. The plants in this study were sampled over a wide latitudinal range, and flowering times vary by latitude (Duncan and Duncan 1987). If the period of overlap is higher in southern populations than the populations farther north, it could explain the higher levels of gene flow in those locations. In addition, *I. glabra* is much more abundant in the south, whereas *I. coriacea* is patchily distributed throughout its range. Even if the degree of overlap in blooming period does not vary among locations, the higher relative abundance of *I. glabra* in the south could increase interspecific pollination relative to intraspecific pollination in *I. coriacea.*

Both *I. coriacea* and *I. glabra* are dioecious and pollinators are required for reproduction (Galle 1997). Abundances of pollinators are known to vary both spatially and temporally among plant populations (Herrera 1988; Schemske and Horvitz 1990; Ashman and Stanton 1991; Eckhart 1992; Cane and Payne 1993; Moeller 2005) due to

different geographic ranges of pollinators relative to the plants they pollinate, yearly fluctuations in pollinator population sizes, and variation in the availability of other sources of pollen or nectar in a given location (Thompson 1988; Eckhart 1992; Moeller 2006). Pollinators also vary in their effectiveness at transferring pollen (Primack and Silander 1975; Schemske and Horvitz 1984; Eckhart 1992). Localized adaptation of floral phenotypes in response to varying communities of pollinators could select for floral traits specialized to specialist pollinators (Schemske and Bradshaw 1999; Schluter 2000; Coyne and Orr 2004; Ellis and Johnson 2009), but could also result in increased variation to allow a greater number of generalist species to pollinate flowers. If pollinator communities vary greatly in space and time, interspecific hybridization rates could vary accordingly.

Hybrid plants have been shown to be less affected by competition than their parental species (Campbell and Snow 2007; Rose et al. 2009). I observed that surrounding vegetation differed among locations: Southern habitats often consisted of very large populations of *I. glabra* and *Serenoa repens* (Bartr.) Small (saw-palmetto) in the understory of long-leaf and slash pine forests. More northern populations had smaller patches of both *I. glabra* and *I. coriacea*, and were outside the range of *S. repens* (McNab and Edwards Jr. 1980). If hybrid plants have better competitive ability against *S. repens* or other sympatric species relative to the parental species, it could potentially explain why hybridization rates were higher in southern populations.

In addition, or in contrast, to environmental conditions, variation in gene flow can be due to evolutionary history or intrinsic genetic incompatibilities. I have no data on the historical distributions of *I. coriacea* and *I. glabra*, but it is reasonable to surmise that

I. glabra could have been driven south during the Pleistocene (Davis 1981; Delacourt and Delacourt 1984). A longer period of sympatry between *I. coriacea* and *I. glabra* in southern populations relative to the more northern distribution would increase the chance that hybridization would occur in the southern part of the distribution.

Intrinsic genetic incompatibilities due to chromosomal rearrangements, differences in ploidy levels, or genic incompatibilities, either within or between loci (reviewed in Coyne and Orr 2004) could also cause gene flow to vary. Most *Ilex* species, including *I. glabra* are diploid (2N = 40) with a few cases of tetraploidy (Frierson 1959; Galle 1997), but the ploidy level of *I. coriacea* remains unknown. I saw no difference in the number of peaks seen in the samples of *I. coriacea* relative to *I. glabra*, suggesting that *I. coriacea* is also diploid. Based on phylogenetic studies of the Aquifoliaceae, reproductive barriers are weak in general among *Ilex* species, and both closely related and distantly related species show signs of hybridization (Manen et al. 2010). Incompatibility can vary with the particular genotypes present in a given location, as well as the species present. Phylogenetic work based on plastids places these species with other North American species whereas nuclear phylogenies place *I. coriacea* and *I. glabra* in a different clade, suggesting at least some degree of introgression with other North American species (Manen et al. 2010). Although not tested here, *I. ambigua* (Michx.) Torr.*, I. cassine* L., *I. decidua* Walter, *I. opaca* Aiton, and *I. vomitoria* Aiton are found within an insect's cruising range of *I. coriacea* and *I. glabra*, and could potentially hybridize with either or both species, further complicating the phylogenetic relationship between *Ilex* species.

Morphological identification

Leaf morphology can be used to identify hybrid samples, but there were no easily identifiable characters that could consistently discriminant hybrids from the parental species. Although the discriminant function based on the full dataset performed quite well, five samples were misclassified whereas functions derived from subsets based on geographic proximity and genetic similarity were able to correctly classify all individuals as defined by their genotype. Varying morphology among regions is not surprising given the genetic structure among locations in each species. However, regional differences complicate matters for identifying and removing hybrid individuals from phylogenetic reconstruction. If hybridization commonly varies among locations and the traits used for phylogenetic reconstruction vary accordingly, not only multiple individuals, but multiple individuals from multiple locations will be needed to correctly identify hybrid status.

The difficulty identifying hybrids from the full dataset is likely due to the wide variation of morphologies found just among these seven hybrid samples. No characters showed consistent differences between hybrid plants and parental species. Much like patterns seen in the hybrid species *Ilex* x *wahlodensis* (Lee et al. 2006; Joung et al. 2011) and hybrids seen between *Brahea dulcis* and *B. nitida* (Ramirez-Rodriguez et al. 2011), introgression appeared primarily unidirectional, with the majority of hybrid individuals identified as varying degrees of backcrosses to *I. coriacea*, and these individuals tended to have a combination of intermediate and *I. coriacea*-like traits. The most interesting comparison was that of the F_1s: one had all intermediate character states, and the other had the majority of transgressive character states as well as intermediate states and ones like one or the other of the parental species. Hybrid F_1s are typically expected to have

intermediate morphology, whereas F_2 individuals are expected to have higher numbers of transgressive traits due to recombination (Whitham 1989; Bangert et al. 2006). I did not test for the F_2 category in NewHybrids as it can be tough to differentiate from F_1's particularly when hybridization rates are low, but the morphology suggests that PHUNGE05 may be an F_2 individual. If so, it could suggest higher rates of hybridization in western Florida than other locations, particularly given the smaller sample size taken from that location.

Patterns of introgression

When the traits were combined there was one generality: hybrid individuals tended to look more like *I. coriacea* than *I. glabra*, matching the patterns of genetic introgression. Four of the five non-F_1 hybrids were identified as being backcrossed to *I. coriacea*, indicating asymmetrical bidirectional introgression of primarily *I. glabra* alleles into the *I. coriacea* background. Similar patterns have been found in cottonwoods, where phytochemical composition and arthropod communities on hybrid trees are most similar to the most genetically similar parental species (Bangert et al. 2006).

There are several potential explanations for the asymmetric introgression of alleles from *I. glabra* into the *I. coriacea* genetic background. First, the abundance of *I. glabra* is much larger than *I. coriacea*, both within a given location and with a larger overall geographic range. Abundance is expected to affect frequency of introgression, where the frequency of alleles from the more abundant species in the genetic background of the less abundant species should be higher than vice versa (Nason et al. 1992; Carney et al. 2000; Burgess et al. 2005). Although F_1 individuals are more likely to backcross to the more common species, backcrosses to the less common species will be easier to

genetically discern from parental and F_1 individuals, resulting in asymmetrical introgression. Because *I. glabra* is more abundant than *I. coriacea*, abundance alone could explain the higher number of individuals identified as backcrosses to *I. coriacea*.

In addition to differences in abundance, at least some degree of allochrony exists between *I. coriacea* and *I. glabra* (Godfrey 1988). If the temporal divergence is heritable, the blooming period of F_1 hybrids could have greater overlap with *I. coriacea* than *I. glabra*. Range expansions are also expected to show patterns of unidirectional introgression from the local species into the invading species (Currat et al. 2008; Excoffier et al. 2009). If *I. glabra* has always had a more northern distribution than *I. coriacea*, and both species were pushed further south during the Pleistocene, when the species moved back north during post-glaciation, *I. coriacea* could have moved into the current range, still occupied by *I. glabra*.

Fitness differences can also affect the directionality of introgression. Alleles conferring increased fitness are expected to introgress more often than neutral or deleterious alleles (Barton 2001; Borge et al. 2005; Whitney et al. 2006). *Ilex glabra* tolerates a wider range of ecological conditions than *I. coriacea* including soil texture, calcium carbonate, pH, salinity, and temperature (USDA 2012), and is also more tolerant of dry conditions (Mohlenbrock 1976; Brooks et al. 1993). If competition for space and resources is important, introgression of traits allowing greater tolerance of variation in these conditions would be more likely to allow *I. coriacea* to potentially expand its microhabitat whereas less would be gained by *I. glabra*, matching the observed pattern in these species.

Hybridization not only affects morphological phenotypes, but chemical as well, yielding repercussions that extend to multiple trophic levels (Whitham et al. 1994; Fritz 1999; Fritz et al. 1999; Whitham et al. 1999; Dungey et al. 2000; Hochwender and Fritz 2004; Wimp et al. 2005; Bangert et al. 2006; Bailey et al. 2009; Smith et al. 2011). Genetically more similar plants are more likely to support more similar communities than genetically dissimilar plants (genetic similarity rule, Bangert et al. 2006). Because hybrids have traits of both parental species, they can attract community assemblages from both, resulting in more diverse communities of species (Whitham et al. 1994; Whitham et al. 1999; Dungey et al. 2000; Hochwender and Fritz 2004; Wimp et al. 2005; Bangert et al. 2006; Bailey et al. 2009). In addition, if traits important to interacting organisms such as phytophagous insects are intermediate in hybrids, hybrid individuals can serve as a 'hybrid bridge' allowing the insects to expand their host range (Floate and Whitham 1993). A leaf-mining fly from a highly specialized clade of insects has recently been found to be forming host races on *I. coriacea* and *I. glabra* (Scheffer and Hawthorne 2007, Chapters 1-2), suggesting hybrids could have served as a mechanism for the original host shift. More work is needed to determine whether or not this is the case.

Conclusions

Natural populations of *I. coriacea* and *I. glabra* are hybridizing resulting in primarily unidirectional introgression of *I. glabra* alleles into an *I. coriacea* background. The morphology of leaves can be used to discriminate parental species and their hybrids and perform best when the samples are divided into regional groupings rather than the entire dataset. The improvement is likely due to genotypic differences, as I identified a geographic mosaic of hybridization in these species. Despite the ability to correctly

classify samples using discriminant function analysis, no consistent patterns were found among individual traits in hybrids relative to the parental species; some traits were transgressive, some intermediate, and some similar to parental species, and all three could be found for a single trait or among traits within a single individual. The phenotypic mosaic seen in hybrids makes it difficult to predict how hybrids would affect phylogenetic inference, as it could depend on the individuals sampled, where they are sampled from, and what traits are used.

Table 3.1. Summary of collected samples from each population and site.

			I. coriacea			*I. glabra*		
			2006	**2007**		**2006**	**2007**	
State	**Site**	**Population**	Geno[1]	Geno	Both[2]	Geno	Geno	Both
FL	Apalachicola National Forest	Sopchoppy	0	5	5	0	1	1
		Hunters	0	9	8	0	8	8
	Etoniah Creek State Forest	East V	0	0	0	0	10	3
		Stuck in Sand	0	10	7	0	8	3
GA	Crooked River State Park	Crooked River	0	0	0	0	10	7
SC	Francis Marion National Forest	Big Ocean Bay	10	8	6	3	10	4
		Wambaw Trail	10	10	9	2	10	6
NC	Croatan National Forest	Catfish Lake	3	7	3	0	9	8
		Road 152	12	11	8	1	9	6
VA	Great Dismal Swamp National Wildlife Refuge	Great Dismal Swamp	0	9	8	0	9	8
DE	Cape Henlopen State Park	Cape Henlopen	0	0	0	0	8	8
		Total	35	69	54	6	92	62

[1]Geno: Genotyped; [2]Both: In addition to genotyping, leaf samples were morphologically measured and analyzed.

Table 3.2. AFLP primer sequences.

Primer[1]	Sequence
*P*AC	5’ - GAC TGC GTA CAT GCA GAC - 3’
*P*AG	5’ - GAC TGC GTA CAT GCA GAG - 3’
*P*AT	5’ - GAC TGC GTA CAT GCA GAT - 3’
*E*ACA	5’ - /56-FAM/GAC TGC GTA CCA ATT CAC A - 3’
*E*AGA	5’ - /56-FAM/GAC TGC GTA CCA ATT CAG A - 3’
*E*ACT	5’ - /56-FAM/GAC TGC GTA CCA ATT CAC T - 3’
*E*AGT	5’ - /56-FAM/GAC TGC GTA CCA ATT CAG T - 3’

Note: Four primer pairs were used: *E*ACA-*P*AG, *E*AGA-*P*AT, *E*ACT-*P*AC, and *E*AGT-*P*AC. [1]*E*: EcoRI; *P*: PstI.

Table 3.3. Landmarks of leaf shape for comparisons of *Ilex coriacea* and *I. glabra*.

Landmark #	Description
1	Base of the petiole
2	Where blade joined petiole (base of blade)
3	¼ length of blade along the midvein
4	Top edge of leaf at 90° angle to the midvein at point 3
5	Bottom edge of the leaf at 90° angle to the midvein at point 3
6	½ length of blade along midvein
7	Top edge of leaf at 90° angle to midvein at point 6
8	Bottom edge of leaf at 90° angle to midvein at point 6
9	¾ length of blade along the midvein
10	Top edge of leaf at 90° angle to midvein at point 9
11	Bottom edge of leaf at 90° angle to midvein at point 9
12	Apex of blade

Note: Top and bottom edges are defined with the apex of the blade to the left of the base of the blade.

Table 3.4. Qualitative measurements of leaves and character coding for *Ilex coriacea* and *I. glabra*.

Apex Angle	
0 – Acute	1 – Obtuse
Apex Shape	
0 - Cuneate (no significant curvature)	1 - Convex (curves away from midvein)
2 - Complex (more than one inflection point)	
Base Angle	
0 – Acute	1 – Obtuse
Base Shape	
0 - Cuneate (no significant curvature)	1 - Convex (curved away from the midvein)
2 - Concave (curved toward the midvein)	3 - Convex on one side and concave on the other
Blade Shape	
0 - Elliptic (widest part of blade in middle 1/5 of long axis)	
1 - Obovate (widest part of blade in the apical 2/5 of long axis)	
2 - Oblong (widest part of blade in middle 1/3 of long axis with opposite margins roughly parallel	
Extent of Teeth	
0 – No teeth	1 - Apex (apical ¼ of margin of blade)
2 - Half (along apical ½ of margin of blade)	3 - Much (beyond apical ½ of margin towards base)
Laminar Symmetry	
0 – Symmetrical	1 – Asymmetrical at base
2 – Asymmetrical at apex	3 – Asymmetrical at both apex and base
Leaf Margin	
0 – Entire (smooth)	1 – Crenate lacking bristle
2 – Crenate with bristle	3 – Bristle only

Table 3.5. Quantitative measurements of leaves from *Ilex coriacea* and *I. glabra*.

Apex Angle	The angle between landmarks 10, 12, and 11
Base Angle	The angle between landmarks 3, 2, and 4
Area	Area of leaf, including petiole
Lower Teeth	Number of teeth along lower leaf margin
Upper Teeth	Number of teeth along upper leaf margin
Length of Blade	Distance between landmarks 2 and 12
Perimeter	Perimeter of leaf including the petiole
Width at ¼ Blade	Distance between landmarks 4 and 5
Width at ½ Blade	Distance between landmarks 7 and 8
Width at ¾ Blade	Distance between landmarks 10 and 11

Note: See Figure 3 and Table 3 for placement of landmarks.

Table 3.6. Summary statistics for AFLPs.

Pop	n	#poly. loci	H_J	H_T	H_S	F_{ST}
Ilex coriacea	105	407	0.16386	0.1840	0.1745	0.0518
I. glabra	97	421	0.17798	0.1940	0.1884	0.0290
Total	202	427	0.20208	0.2518	0.1709	0.3210

Notes: n: number of samples; # poly. loci: number of polymorphic loci; H_J: Nei's genetic diversity; H_T: total gene diversity; Nei's H_S; Wright's F_{ST}.

Table 3.7. Analysis of molecular variance estimated using the ADONIS function for AFLP data from *Ilex coriacea* or *I.glabra*.

	Source	d.f.	SS	MS	F - model	R^2	P (>F)
a)	Location	6	4.00572	0.66762	5.52539	0.0982	**< 0.0005**
	Species nested in Location	5	13.82695	2.76539	22.88704	0.3390	**< 0.0005**
	Residuals	190	22.95728	0.12083	. - .	0.5628	. - .
	Total	201	40.78996			1	
b)	***Ilex coriacea***						
	Location	4	1.44967	0.36242	3.53747	0.1240	**< 0.0005**
	Residuals	100	10.24510	0.10245	. - .	0.8760	. - .
	Total	104	11.69477			1	
c)	***Ilex glabra***						
	Location	6	1.67066	0.27844	1.97132	0.1162	**< 0.0005**
	Residuals	90	12.71218	0.14125	. - .	0.8838	. - .
	Total	96	14.38284			1	

Notes: Variation was partitioned (a) among species nested within each location, then within each species among locations (b, c).

Table 3.8. Estimates of pairwise F_{ST}.

a)

	VA	NC	SC	East FL	West FL
VA	--	0.0284	0.0022 *	< 0.0001 *	< 0.0001 *
NC	0.0250	--	< 0.0001 *	0.0008 *	0.0008 *
SC	0.0428	0.0365	--	0.0006 *	0.0004 *
East FL	0.0923	0.0610	0.0522	--	0.0578
West FL	0.0752	0.0614	0.0465	0.0246	--

b)

	DE	VA	NC	SC	GA	East FL	West FL
DE	--	0.0154	0.0062	0.0402	0.0074	0.0052	0.0032
VA	0.0344	--	< 0.0001 *	0.0516	0.0132	0.0056	< 0.0001 *
NC	0.0346	0.0569	--	0.0004 *	0.0006 *	< 0.0001 *	< 0.0001 *
SC	0.0233	0.0144	0.0281	--	0.1138	0.0070	0.0252
GA	0.0375	0.0125	0.0396	0.0083	--	0.1214	0.0542
East FL	0.0377	0.0229	0.0523	0.0192	0.0060	--	0.0594
West FL	0.0528	0.0400	0.0572	0.0227	0.0097	0.0106	--

c)

	VA	NC	SC	East FL	West FL
VA	--	0.0214	0.1238	0.0298	0.1138
NC	0.0031	--	0.0674	0.0014 *	0.0360
SC	0.0079	0.0069	--	0.0026 *	0.1116
East FL	0.0203	0.0400	0.0324	--	0.0194
West FL	0.0101	0.0161	0.0071	0.0214	--

Notes: a) Among sampling locations of *Ilex coriacea*. b) Among sampling locations of *I. glabra*. c) Among sampling locations with combined *I. coriacea* and *I. glabra*. F_{ST} is below the diagonal and associated p-values based on resampling are above the diagonal. * Significant at $p < 0.5$ after Bonferroni correction for multiple comparisons.

Table 3.9. Assessment of discriminant functions based on samples separated by regions and for all samples combined.

	Efficiency			**Accuracy**			**Type I Error**
	I. coriacea	*I. glabra*	hybrids	*I. coriacea*	*I. glabra*	hybrids	
DE, VA, NC	1	1	1	1	1	1	0
SC	1	1	1	1	1	1	0
GA, East FL, West FL	1	1	1	1	1	1	0
All populations	0.958	0.984	0.714	0.958	0.984	0.714	0.018

Notes: See text for definitions of efficiency, accuracy, and type 1 error. Functions based on locational divisions performed better than the function with all samples combined. Hybrids were most often incorrectly assigned, followed by *I. coriacea*, likely because hybrids more closely resembled *I. coriacea* than *I. glabra*.

Table 3.10. Measurements of quantitative variables in *Ilex coriacea*, *I. glabra*, and their hybrids.

	Apex angle	Base angle	Area (mm^2)	Left teeth	Right teeth	Length of blade (mm)	Perimeter (mm)	Width at ¼ blade (mm)	Width at ½ blade (mm)	Width at ¾ blade (mm)
Ilex coriacea	73.81 ± 10.08°	64.54 ± 9.83°	912.83 ± 320.61	2.65 ± 1.90	2.58 ± 1.80	53.64 ± 10.66	148.19 ± 28.22	17.06 ± 4.20	24.22 ± 4.85	20.32 ± 4.16
Ilex glabra	70.87 ± 10.48°	58.24 ± 11.04°	498.13 ± 157.77	1.79 ± 0.90	1.80 ± 0.93	41.46 ± 8.11	112.65 ± 20.94	11.51 ± 2.65	16.56 ± 3.52	14.75 ± 3.04
Hybrid Samples										
P152C288	75.02°	55.77°	802.24	**0** †	1	51.71	142.86	13.59	23.19	19.85
P152CE02	77.70°	65.28°	566.31	3	**4** †	41.95	117.36	13.54	19.84	17.07
PBOBC191	84.67°	77.68°	662.57	**0** †	2	41.23	117.97	**16.74** †	23.31	19.01
PBOBCE05	63.25°	48.81°	499.86	1	3	44.12	125.05	10.25	16.81	14.96
PHUNC012	86.66°	**83.72°** †	723.50	**4** †	1	41.93	120.41	**19.04** ††	**24.65** ††	20.11
PHUNGE05	78.12°	77.17°	**198.12** *	2	1	**23.11** †**	**71.28** †**	9.50	**11.44** **	**9.49** **
PSOPC005	89.58°	**85.54°** *†	**904.29** †	1	0	46.05	131.30	**21.46** ††	**28.29** ††	**23.23** ††

Notes: Means and standard deviations of morphological measurements are listed for each parental species. Hybrid samples are listed individually beneath the parental means. Colored cells represent values with a probability of 90% or less of belonging to a parental distribution based on a t-test to compare a single observation to the mean. Blue represents values outside the distribution of *I. coriacea*, yellow for values outside the range of *I. glabra*, and peach for transgressive traits. Symbols indicate significant differences from parental species. *I. coriacea:* * $p < 0.05$, ** $p < 0.01$; *I. glabra*: † $p < 0.05$, †† $p < 0.01$.

Table 3.11. Character states of qualitative variables in *Ilex coriacea*, *I. glabra*, and their hybrids.

	Apex angle		Apex shape			Blade shape			Base angle		Base shape				Extent of teeth				Laminar symmetry				Leaf margin			
	0	**1**	**0**	**1**	**2**	**0**	**1**	**2**	**0**	**1**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Ilex coriacea	45	3	1	10	37	43	4	1	47	1	16	22	6	4	3	14	8	23	14	6	13	15	3	0	5	40
Ilex glabra	59	2	0	61	0	42	14	5	60	1	18	39	1	3	1	42	17	1	20	19	6	16	1	54	6	0
Hybrids	7	0	0	**2**	**5**	6	1	0	7	0	4	3	0	0	0	**3**	**3**	**1**	2	**2**	0	3	0	**1**	0	**6**

Notes: Yellow represents individuals with character states that more closely resemble those in *I. coriacea* than in *I. glabra*. The reverse is true for boxes highlighted in blue.

Figure 3.1: Endemic range *Ilex coriacea* and *I .glabra* with collection sites labeled.

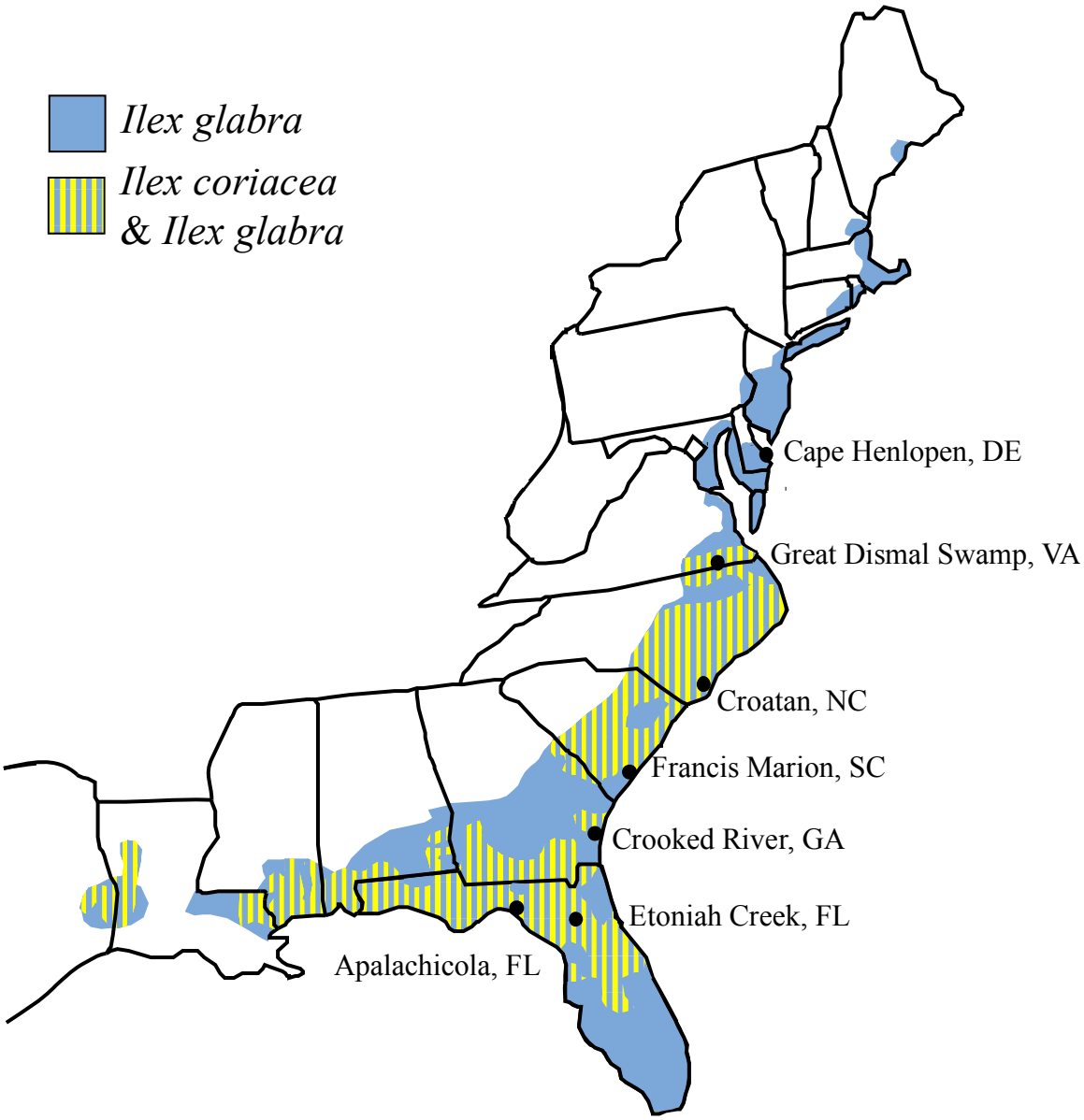


Figure 3.2. Sample scan of leaves from *I. glabra.*

Figure 3.3. Example of landmarks on an *I. coriacea* leaf.

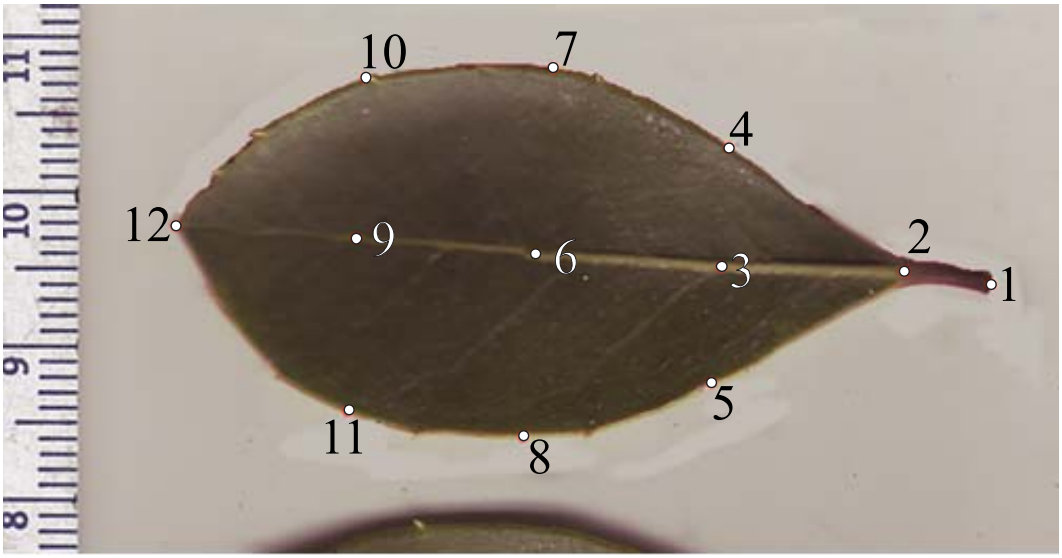


Figure 3.4. F_{ST} between *Ilex coriacea* and *I. glabra* among sampling locations. Error bars are 95% confidence interval estimates for F_{ST}. F_{ST} varies in magnitude among locations, but the differences are not statistically significant.

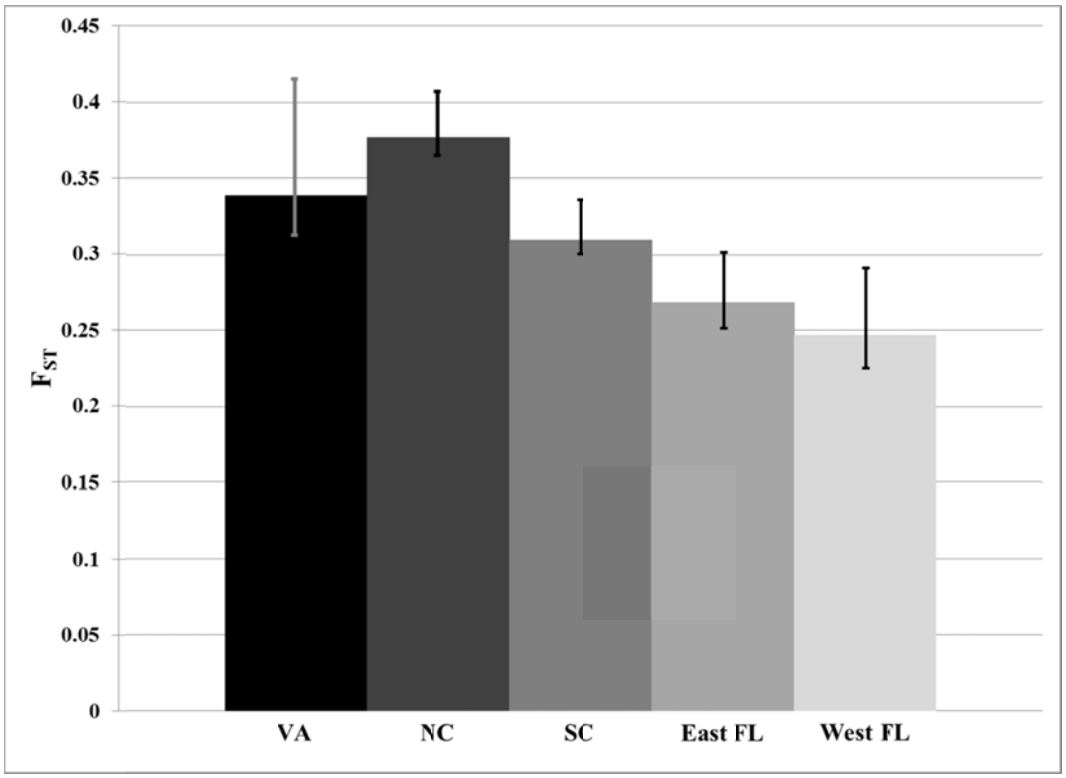


Figure 3.5. ΔK for structure runs using a K of 1 to 15. A K of 2 was most representative of the data

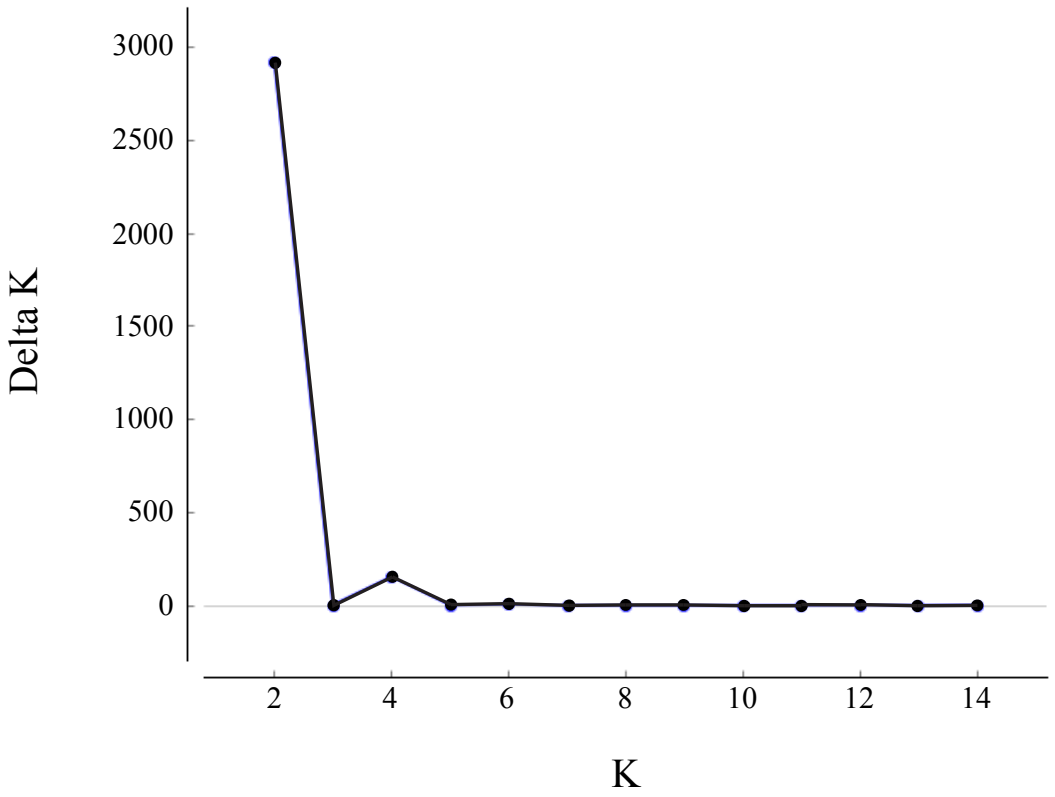


Figure 3.6. Results of STRUCTURE analysis K=2. Yellow corresponds to *Ilex coriacea* and blue to *I. glabra*. Each bar represents a single individual with the portion colored representing the posterior probability of the individual belonging to each cluster. Individuals are ordered by population from north to south (left to right).

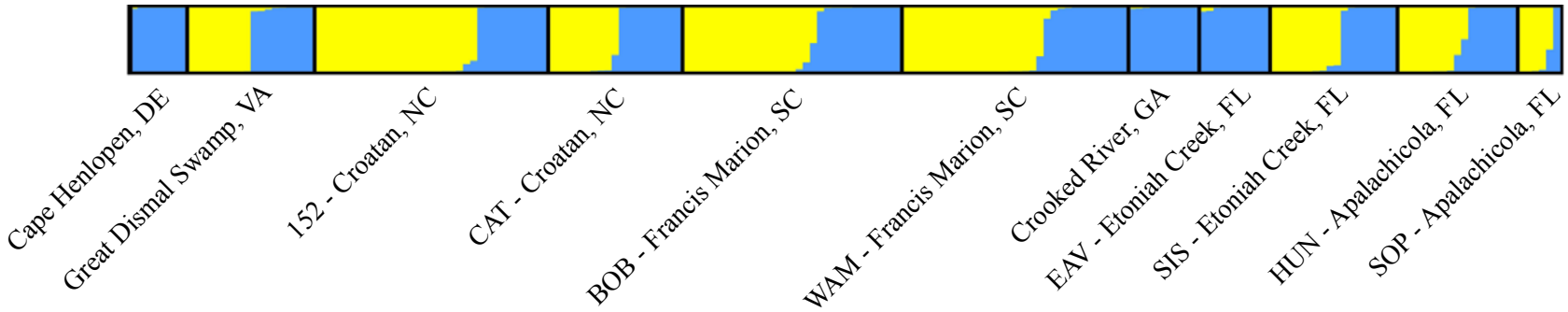


Figure 3.7. Comparison of the frequency of allele presence between *Ilex glabra* and *I. coriacea*. Variation among loci indicates a genetic mosaic of divergence among species in these loci.

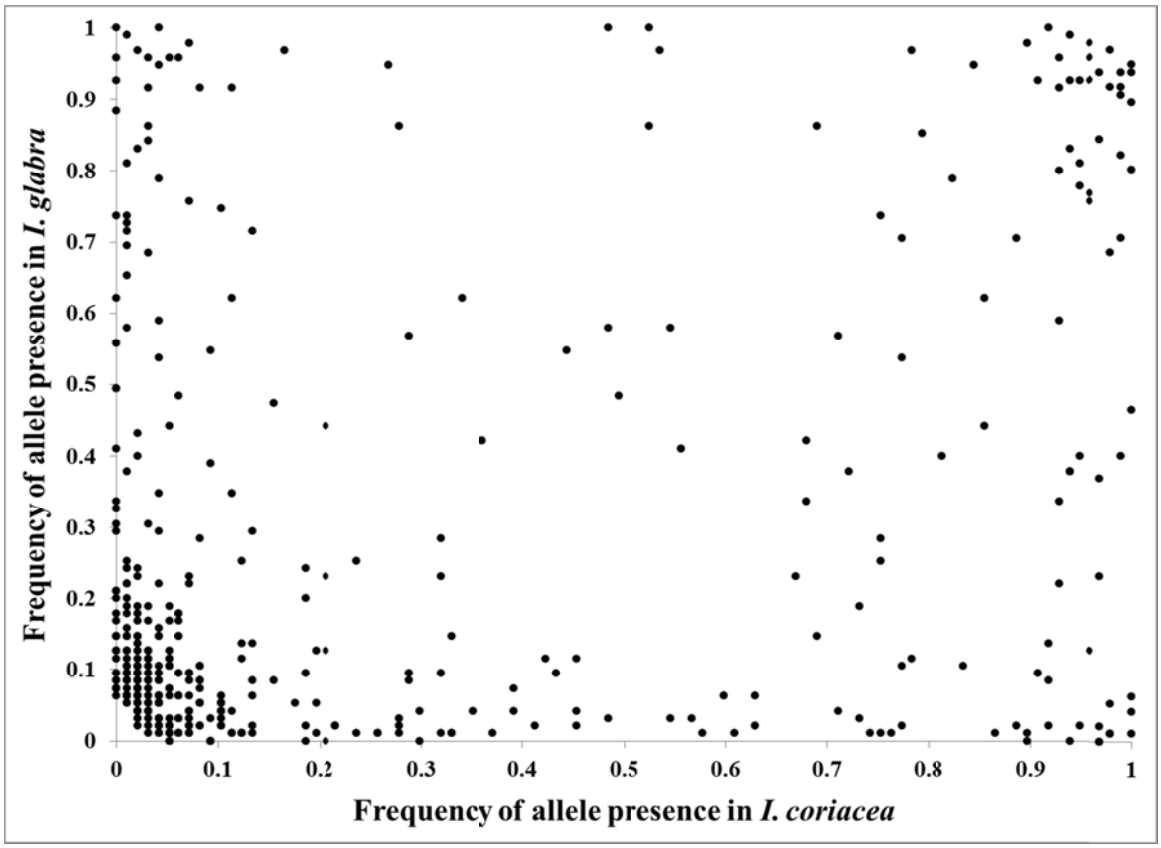


Figure 3.8. Procrustes rotations of landmark data. a) *I. coriacea*, b) *I. glabra*, c) hybrids, d) mean shape of *I. coriacea*, *I. glabra*, and their hybrids superimposed under the same rotation and scaling. Yellow = *I. coriacea*, Blue = *I. glabra*, Green = Hybrids. The mean shape of hybrid leaves appear rounder and broader than those of *I. coriacea* and *I. glabra.*

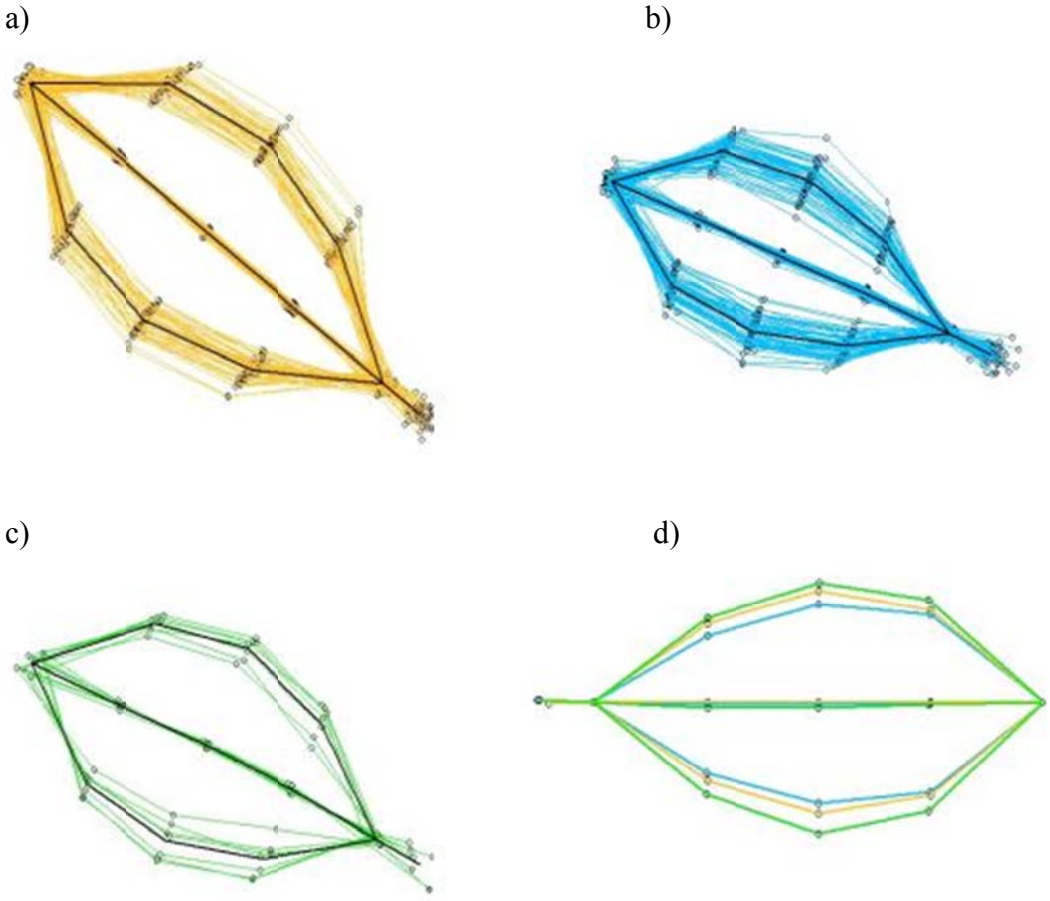
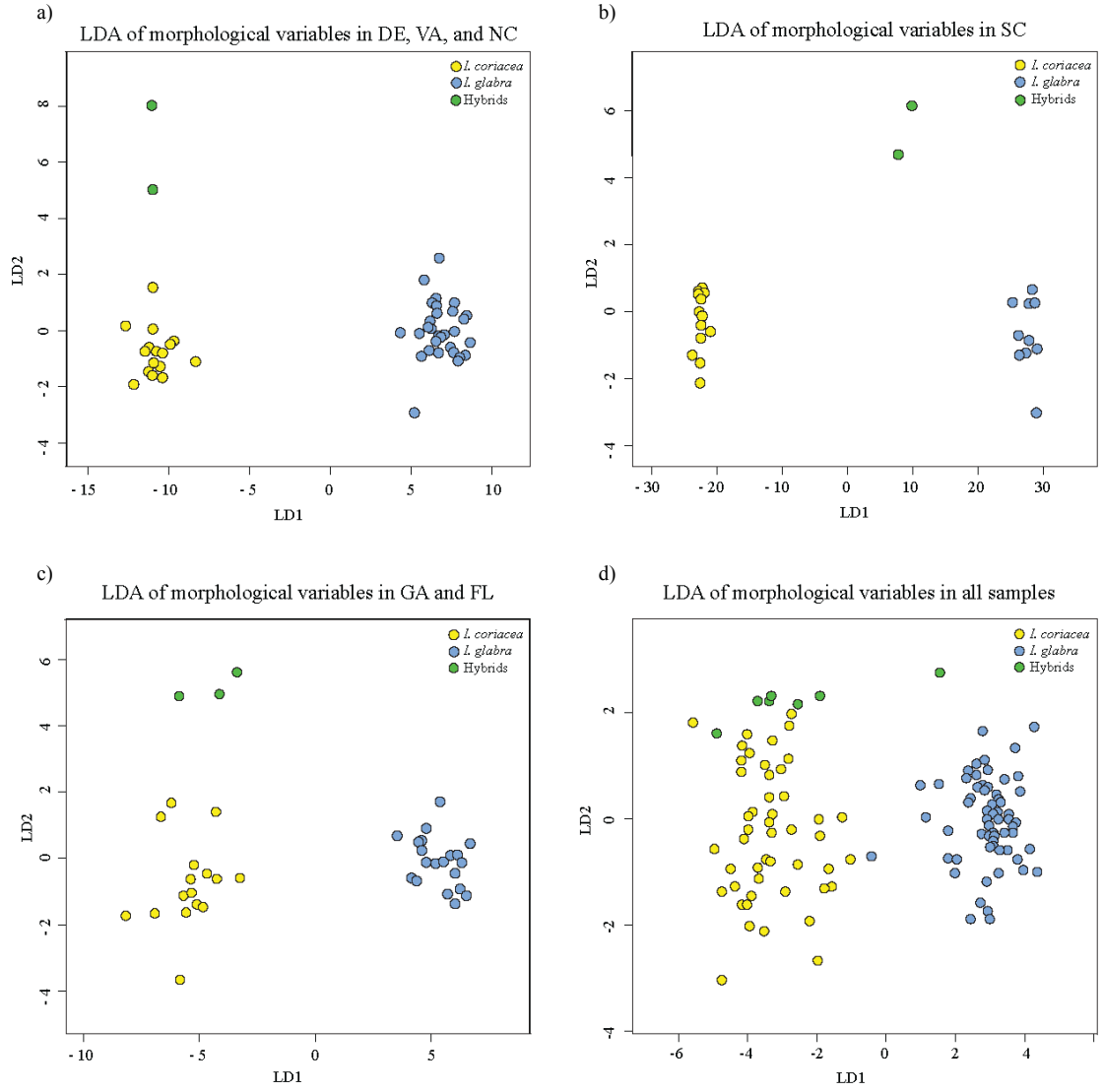


Figure 3.9. Plots of first two linear discriminants from discriminant functions based on traditional morphological characters from samples of *Ilex coriacea*, *I. glabra*, and hybrids. a-c) Analyses of regional divisions of samples. d) Analysis of all samples combined. Regions were chosen based on geographic proximity, genetic similarity, and presence of hybrids. Individuals are plotted according to their taxonomic classification based on analysis of genetic admixture: yellow represents > 90% *I. coriacea*, blue represents > 90% *I. glabra*, and green represents hybrids, all individuals not classified as parental species. The first axis discriminates between parental species and the second axis discriminates the hybrids from parental species. In general, hybrid individuals more closely resemble *I. coriacea* than *I. glabra*.

Figure 3.9


a)
LDA of morphological variables in DE, VA, and NC
I. coriacea
I. glabra
Hybrids
LD2
LD1
b)
LDA of morphological variables in SC
I. coriacea
I. glabra
Hybrids
LD2
LD1
c)
LDA of morphological variables in GA and FL
I. coriacea
I. glabra
Hybrids
LD2
LD1
d)
LDA of morphological variables in all samples
I. coriacea
I. glabra
Hybrids
LD2
LD1

CHAPTER 4: GENE FLOW BEGETS GENE FLOW: TESTING THE HYBRID BRIDGE HYPOTHESIS AND ITS ROLE IN ECOLOGICAL SPECIATION

ABSTRACT

Interspecific hybridization is common in plants and is known to affect plant resistance to herbivory either positively or negatively depending on the herbivore in question and the phenotype of the hybrids. The relative resistance of hybrids will affect the ability of herbivores to colonize hybrids and move between parental species. When populations of herbivores on different host plant species are genetically differentiated from one another, hybridization between the host plant species could affect how much gene flow occurs between the host forms. Here, I demonstrate that hybrid plants may be serving as 'hybrid bridges' to host forms of a leaf-mining fly, *Phytomyza glabricola* on its two holly hosts, *Ilex coriacea* and *I. glabra*. Hybridization between host plant species and the amount of gene flow between host forms of the fly vary among different locations. As hybridization rates of populations of its host plants increase, so does gene flow between host forms of the insect. Considering the number of plant species that hybridize, it is likely that hybrid bridges and barriers are important for many host form and host race systems. In addition, the presence of hybrid bridges indicates hybrid plants may allow host range expansion in specialized herbivores. Hybrid bridges may be the missing link explaining how adaptive radiations proceed in specialized lineages of herbivorous insects.

INTRODUCTION

Natural interspecific hybridization, successful matings in nature between two species, occurs in an estimated 25% of plant species (Ellstrand et al. 1996; Arnold 1997; Rieseberg 1997; Mallet 2005). First generation hybrids often share similarities with either parental species depending on the particular combination of alleles passed on from each parent (Lexer et al. 2009), but will also display intermediate and transgressive phenotypes (Rieseberg and Ellstrand 1993; Rieseberg et al. 1999). The resulting genotypic and phenotypic mosaics continues to increase with recombination in advanced generation hybrids (Carney et al. 2000; Travis et al. 2008), particularly between taxa with porous genomes (Lexer et al. 2009). These changes in genotypic and phenotypic diversity not only influence the ecology and evolution of the hybridizing species, but also affect communities of species affiliated with the hybridizing plants (Hochwender and Fritz 2004).

A number of studies indicate hybridization can alter plant resistance to herbivorous species, directly and indirectly changing the abundance and community structure of phytophagous species (Whitham et al. 1994; Fritz 1999; Fritz et al. 1999; Whitham et al. 1999; Dungey et al. 2000; Hochwender and Fritz 2004; Wimp et al. 2005; Bangert et al. 2006; Bailey et al. 2009; Smith et al. 2011). Whether hybridization will result in an increase or decrease in species abundance and diversity depends on the particular combination of traits displayed in individual plant hybrids (Bailey et al. 2009). Changes in phenology (e.g., flowering date (Johnson and Agrawal 2005) and budburst (Hunter et al. 1997)), defensive chemistry (e.g., tannins (Bailey et al. 2006), glucosinate (Clauss et al. 2006), and flavonoids (Johnson et al. 2009)), defensive morphology (e.g.,

trichome density (Clauss et al. 2006; Johnson 2008) and wax (Zalucki et al. 2002)), and nutritive quality (e.g., leaf water content, percent nitrogen (Strong et al. 1984; Huberty and Denno 2006; Johnson 2008)) can all impact the preference, performance, and distribution of beneficial and herbivorous species (Fritz 1999; Fritz et al. 1999; Orians 2000; Carmona et al. 2011).

Depending on the fitness of herbivorous species on hybrid plants relative to the parental plant species, hybrids can serve as bridges (Floate and Whitham 1993) or barriers to movement between plant species. If specialized herbivores have moderate fitness on hybrids relative to the natal host plant species, but low fitness on the alternate parental plant species, hybrid plants can serve as ecological and evolutionary bridges allowing the herbivores to gradually adapt to the non-natal species ('hybrid bridge hypothesis'; Floate and Whitham 1993; Whitham et al. 1999). If, on the other hand, herbivores have low to zero fitness on hybrid plants, natural selection should favor avoidance of hybrid plants, potentially resulting in reproductive isolation between populations of herbivores associated with each parental species (Barton 2001).

Although plant hybridization has received little attention in regards to the evolution of host-associated differentiation (Dres and Mallet 2002), hybrid bridges and barriers could affect the degree of specialization and genetic divergence among host forms. The role of host plant hybridization in host-associated differentiation, and potentially speciation, has not been tested partially due to a lack of appropriate systems. An effective method requires sympatric host plant species, hybridization between the hosts, and herbivorous species specialized on one or both of the parental plant species.

In this study, a native species of leaf-mining fly, *Phytomyza glabricola* Kulp, and its two native holly hosts, *Ilex coriacea* (Pursh) Chapman and *I. glabra* (Linnaeus) Gray were used to study the effects of host plant hybridization on genetic distance in insect host forms, host-associated populations where the kind and degree of host-associated variation have not been fully examined (Funk 2012). *Phytomyza* (Diptera: Agromyzidae) is a large genus (> 400 species) of flies mainly composed of monophagous leaf-miners (Spencer et al. 1986; Spencer 1990). *Phytomyza glabricola* belongs to a radiation of 14 closely related species, all of which feed on hollies in the genus *Ilex,* and most of which are monophagous (Kulp 1968; Scheffer and Wiegmann 2000; Lonsdale and Scheffer 2011). In contrast, *P. glabricola* feeds on two native species of holly, the ancestral host, *I. glabra*, and *I. coriacea*, which are sympatric for much of their range (Scheffer 2002; Chapters 1-3). The adults from each host do not appear to differ morphologically in either external characters or genitalia (Scheffer 2002). The leaf-miners do, however, differ in development time, taking nine months to develop on *I. coriacea* versus two to four weeks on *I. glabra* (Kulp 1968; Al-Siyabi and Shetlar 1998; Scheffer 2002). Despite differences in development time among host plant species, adult *P. glabricola* emerge in synchrony in mid-January to mid-February (Scheffer 2002).

The host plants of the leaf-miners, *I.coriacea* and *I. glabra*, are members of the family Aquifoliaceae (hollies), that consists of a single extant genus (Powell et al. 2000), *Ilex* (L.) of approximately 600 species (Loizeau et al. 2005). The two species are evergreen shrubs native to pocosins, hammocks, baygalls, and long-leaf pine forests on the coastal plain of the eastern United States (Duncan and Duncan 1987; Godfrey 1988). The more cold tolerant *I. glabra* grows from coastal Nova Scotia south to Florida, and

along the Gulf of Mexico into eastern Texas (Duncan and Duncan 1987; Figure 1). The range of *I. coriacea* is completely encompassed within the range of *I. glabra*, extending from southern Virginia to northern Florida and Texas. Throughout its range, *I. coriacea* is sympatric and often syntopic with *I. glabra* (Scheffer 2002; Chapters 1-3), the more abundant of the two species (Mohlenbrock 1976; Richardson 1983; Brewer 1998; Brockway and Lewis 2003; Clark et al. 2008). The two are likely sister species (Manen et al. 2010) and hybridize in the wild (Chapter 3). Although hybridization rates are consistently low, they do vary among locations (Chapter 3).

No-choice mating trials have revealed that *P. glabricola* from the same host plant will mate, oviposit, and develop on either holly species but cross-host mate pairs failed to produce offspring (Chapter 1). The reproductive isolation seen in the mating trials is also expressed as host-plant based genetic structure (Scheffer and Hawthorne 2007), and genome scans of the flies show signs of divergent selection, suggesting they may be in the midst of ecological speciation (Chapter 2). The combination of sympatry throughout the range of *I. coriacea*, natural variation in rates of hybridization among the *Ilex* species, and the presence of host forms in *P. glabricola* allow me to test whether hybrid plants serve as a bridge or barrier for these flies. To examine the relationships between host plant hybridization and gene flow in the insects, I will focus on population-level and individual comparisons of flies and their host plants. I ask how does genetic divergence in host forms of *P. glabricola* change in relation to the degree of hybridization among *I. coriacea* and *I. glabra*? Previous work has demonstrated a geographic mosaic of hybridization and phenotypic divergence among locations of the two *Ilex* species (Chapter 3) and a geographic mosaic of genetic divergence among host-associated

populations of flies (Chapter 2), allowing for population-level comparisons of gene flow between fly populations and gene flow between holly species. In addition, flies were collected as pupae within their leaf-mine allowing for direct genetic comparison of the fly and its host plant.

The relationship between genetic divergence in the flies and hybridization in the host plants will depend on the hybrid phenotypes for traits that affect host plant use in the flies. If these traits are intermediate in hybrids, host forms of *P. glabricola* specialized to each parental species could encounter one another on hybrid plants, potentially increasing gene flow and decreasing genetic divergence between host forms (Floate and Whitham 1993; Gange 1995). If so, I expect a positive relationship between gene flow in the plants and gene flow in the insects (Figure 4.2). On the other hand, if hybrid plants have novel or transgressive traits rendering them unpalatable to the flies, I expect hybrids to serve as 'barriers', increasing genetic divergence between host forms, resulting in a negative relationship between gene flow in the plants and gene flow in the insects. Finally, there could be no relationship at all between hybridization in host plants and gene flow in insects because of a phenotypic mosaic of important traits, some of which may attract flies from either parental host plant species and some of which may deter flies.

METHODS

Collections

Leaf-mines and leaves were collected in January through March of 2006 from Croatan National Forest, NC and Francis Marion National Forest, SC, and again in 2007 with additional samples from Cape Henlopen State Park, GA, the Great Dismal Swamp National Wildlife Refuge, VA, Crooked River State Park, GA, Etoniah Creek State

Forest, FL, and Apalachicola National Forest, FL (Figure 4.1, Table 4.1). *Ilex glabra* was found at every site, however *I. coriacea* was not found at two sites (DE and GA), the first of which is outside the known geographic range of *I. coriacea*. Both years, leaves containing well-developed leaf-mines and visible larvae were removed from host plants and placed into plastic bags labeled with site, date, and putative host plant species. In 2007, in addition to the leaf-mines, the stem closest to the base of the plant with at least five leaves and no new growth was removed from each plant and placed with the collected leaf-mine, if present, or into its own plastic bag labeled with site, date, and putative species if no leaf-mine was present on the plant. Pupae were later dissected from the leaf-mines and placed individually in 0.5 mL Eppendorf tubes and stored in a moist chamber until the emergence of adults, at which point adult flies were placed in 100% ethanol and stored at -80°C. After dissection of mines, leaves were placed in labeled coin envelopes and stored at -80°C.

AFLPs

A total of 202 plants (97 *I. coriacea*, 95 *I. glabra*, and 10 hybrids) and 183 flies (96 from putative *I. coriacea*, and 87 from putative *I. glabra*) were genotyped using AFLPs. Methods were as described in Chapters 2 and 3. Briefly, all samples were genotyped using four primer pair combinations (Chapters 2, 3). PCR products were separated with an ABI 3730 DNA Analyzer (Applied Biosystems, Carlsbad, CA) using MapMarker X-Rhodamine (ROX) labeled 1000bp ladder (BioVentures, Murfreesboro, TN). Electropherograms were scored using either GENEMAPPER v.3.7 (Applied Biosystems, Carlsbad) or GENEMARKER (Soft Genetics, LLC, State College, PA) for the flies and plants, respectively. Six individuals were replicated across plates, and ten

individuals within plates, to test for repeatability. In addition, negative controls (H_2O template) were run for every step of the process. After scoring, loci with peaks in the negative controls were removed from the analysis, as were loci with peak mismatches among repeated samples. Finally, loci with small fragment frequencies were removed to eliminate any negative correlation of fragment frequency and fragment size that may be caused by excessive homoplasy (Vekemans et al. 2002).

Analyses

The hybrid index was chosen to quantify the degree of hybridization contributing to an individual sample. The hybrid index is an allele-frequency based estimate of the proportion of alleles in an individual that are inherited from one of two parental populations or species (Buerkle 2005). The index ranges from 0 to 1 where, for this study, 0 represents either flies collected from *I. coriacea* (hereafter "coriacea-flies") or samples of *I. coriacea* and 1 represents either flies collected from *I. glabra* (hereafter "glabra-flies") or samples of *I. glabra*. Reference samples are needed to estimate hybrid indices, therefore samples were classified as parental if the sample had a 0.99 or greater membership in a parental category as assigned by NEWHYBRIDS (Anderson and Thompson 2002; Anderson 2008; see Chapters 2,3 for details). A total of 30 coriacea-flies and 51 glabra-flies, and 84 *I. coriacea* and 89 *I. glabra* were classified as reference samples. Hybrid indices were estimated for each individual using the HINDEX function in the package INTROGRESS (Buerkle 2005; Gompert and Buerkle 2009, 2010) using the statistical package R (R Development Core Team 2010). Although codominant markers are preferred for estimating the hybrid index (Buerkle 2005), dominant markers can be used if enough markers are used with divergent allele frequencies in parental species (van

Loo et al. 2008; Bellusci et al. 2010; MacKay et al. 2010; Vereecken et al. 2010; Hrsak et al. 2011; Xu et al. 2011).

The resulting hybrid indices were then used in two ways. Direct comparisons were made between the hybrid index of individual flies and plants if both the fly and plant were successfully genotyped. Individual comparisons allowed visual examination of the distribution of coriacea-flies, glabra-flies, and genotypic intermediates on *I. coriacea*, *I. glabra*, and their hybrids. The presence of both coriacea-flies and glabra-flies, or of intermediates, on hybrid plants would be an indication hybrid plants are serving as a bridge rather than a barrier to gene flow. If, instead, no flies are found on hybrid plants, plants are likely serving as a barrier to gene flow.

Many genotyped samples of plants did not have corresponding genotypes for flies, and vice versa (Table 4.1), therefore hybrid indices were combined to perform population-level comparisons. Rather than using F_{ST}, which eliminates much of the information regarding introgression of alleles, mean hybrid index scores were calculated for each population. Previous work with mean hybrid index scores were not corrected for parental identification (Bennuah et al. 2004; Burgess et al. 2005; Zitari et al. 2012), which could result in misleading averages close to 0.5 for populations with roughly equal numbers of samples from each parental species. Therefore, an adjusted hybrid index was created to estimate the amount of introgression present in a given population. Hybrid index estimates above 0.5 were subtracted from 1 to normalize the data (Figure 4.3) resulting in a value of 0 for parental individuals and values between 0 and 0.5 for individuals with mixed ancestry. For populations with a minimum of 5 individuals, the

adjusted hybrid indices were averaged across all individuals within a given population to get an estimate of the degree of introgression for that population.

A linear regression was used to test whether the mean adjusted hybrid index of fly populations depends on the mean adjusted hybrid index of plant populations. Holly species are much longer lived than the leaf-miners that feed on them, and are more likely to affect gene flow of the flies than vice versa. The flies are not typically present during the blooming periods of their host plants, and are therefore not likely pollinators of the holly species. If hybrid plants are serving as a hybrid bridge, then more flies should be moving between host plants, resulting in a positive relationship between hybridization in plants and gene flow in the flies (Figure 4.2a). If, instead, hybrid plants are serving as a hybrid barrier to gene flow, then greater hybridization in plants should result in lower gene flow among host-associated populations of flies (Figure 4.2b). A non-significant correlation would indicate no relationship between hybridization in the plants and hybridization in the flies (Figure 4.2c). The linear regression was estimated using the function LM using the statistical package R (R Development Core Team 2010). For regressions of a single variable and the response, the function conducts a generalized linear model based on a Gaussian distribution.

RESULTS AND DISCUSSION

A total of 427 AFLP markers were retained for *I. coriacea* and *I. glabra* and a total of 267 markers for coriacea-flies and glabra-flies (Chapters 2, 3). Markers in the plants ranged from 76-720bp in length, with 79% having a fragment size above 200bp. In the flies, markers ranged from 78-792bp in length with 92% of the markers above 200bp.

Variation in hybrid indices among populations

There was much more variation in the hybrid indices of flies than in the hybrid indices of plants (Figure 4.4, Appendix F, G), which is not surprising given the plants are considered different species, whereas the flies are currently considered host forms (Chapter 2). Previous work revealed reproductive isolation among host forms of *P. glabricola* (Chapter 1), but genetic data suggests the flies are not yet different species and are likely undergoing ecological speciation (Chapter 2). However, if the flies are different species, there has likely not been enough time for lineage sorting to differentiate neutral loci among the species, reflected here as intermediate hybrid index scores.

In both plants and flies, the lowest average adjusted hybrid indices were in Cape Henlopen, as expected as it is outside the range of *I. coriacea*. In addition, both plant and fly populations had the highest scores in western Florida (Sopchoppy and Hunters populations, respectively), suggesting hybridization is much more prevalent in that area. A number of factors could explain the increased adjusted hybrid index in southern populations. Both *I. coriacea* and *I. glabra* are dioecious and pollinators are required for reproduction (Galle 1997). *Ilex glabra* is much more abundant in the south, whereas *I. coriacea* is patchily distributed throughout its range, so the higher relative abundance of *I. glabra* in the south could increase interspecific pollination relative to intraspecific pollination in *I. coriacea*. In addition, southern populations of *I. coriacea* begin blooming weeks before *I. glabra* (Godfrey 1988), but the degree of overlap in blooming time for these species is unknown. If the period of overlap is higher in southern populations than the populations farther north, it could explain the higher levels of gene flow among the plants in those locations.

The surrounding environment also differs across the geographic distribution of *I. coriacea* and *I. glabra*. Populations of *I. coriacea* and *I. glabra* differ in the surrounding plant communities; particularly the presence of *Serenoa repens* (Bartr.) Small (saw-palmetto) in the south. Pollinator communities may also vary spatially and temporally (Herrera 1988; Schemske and Horvitz 1990; Ashman and Stanton 1991; Eckhart 1992; Cane and Payne 1993; Moeller 2005). Differences in competitive ability among hybrid and parental plants against *S. repens* or other sympatric species, or variation in pollinator communities could potentially explain why hybridization rates were higher in southern populations. However, these factors are not likely to directly influence the degree of gene flow among host forms of the flies.

Variation in gene flow can also be heavily influenced by evolutionary history. Although I have no data on the historical distributions of *I. coriacea* and *I. glabra*, it is reasonable to surmise that *I. glabra* could have been driven south during the Pleistocene (Davis 1981; Delacourt and Delacourt 1984). A longer period of sympatry between *I. coriacea* and *I. glabra* in southern populations relative to the more northern distribution would increase the chance that hybridization would occur in the southern part of the distribution, and if the flies have been on the plants for that long of a period, they too may have had more chances for gene flow.

The variation in the degree of host-associated genetic divergence is expected to be influenced by environmental differences among locations, but is more likely due to indirect effects mediated by the host plant than direct effects from the outside environment. For example, flies on *I. glabra* experience multiple generations in a year, whereas flies on *I. coriacea* have only a single generation (Scheffer 2002), and

development time is influenced by the environment (Chapter 1), meaning higher temperatures and increased daylight hours in the south could increase developmental rates of flies in these locations. The additional generations in flies emerging from *I. glabra* would allow selection to more efficiently eliminate slightly deleterious alleles and increase the probability that any hybrid flies present would backcross to glabra-flies, increasing genetic divergence among host forms of the flies. However, I see decreased genetic divergence in southern populations, suggesting differences in development time are not the driving force underlying differences in genetic divergence. Instead, if hybrid plants are influencing the rate of gene flow among host forms of the insects, such as serving as a hybrid bridge, then the increased hybridization rates in the south could allow host forms of the flies to encounter one another via hybrid plants regardless of the cause of variation in hybridization rates among *I. coriacea* and *I. glabra*.

Hybrid bridge

A significant positive relationship was found between the average adjusted hybrid indices of flies and plants among locations with both host forms ($R^2 = 0.6717$, $F_{1,4} = 8.182$, $p = 0.04591$; Figure 4.5). The averages from CHE were not included in the regression analysis because they are outside the range of *I. coriacea*, but serve as a control because they should have the lowest hybrid indices (Figure 4.5). Populations of plants with the highest scores (and therefore the highest hybridization rates) had the highest rates of gene flow in the flies, and the same was true for the lowest scores (Figure 4.5), matching the expectation for hybrids serving as bridges for flies among host plant species. The correlation is primarily driven by the Hunters population from western Florida, (without HUN, $R^2 = 0.1313$, $F_{1,3} = 0.4535$, $p = 0.5489$), but I believe that point is

valid. Plants from populations in Florida are more difficult to identify than plants in the northern populations (JBH, S. J. Scheffer*, personal observation*), suggesting higher hybridization rates in the south (Chapter 3). Had I sampled from additional southern populations in Alabama, Louisiana, and Mississippi as well as further out the panhandle of Florida, I would have likely found average hybrid indices at or above the level found in Hunters.

It was much harder to find a specific pattern from comparisons of individual flies and their hosts, mainly due to low numbers of hybrid plants (Chapter 3), and even lower numbers that also had corresponding genotyped flies (Table 4.1). Of the two plants identified as hybrids, one was associated with a fly with mixed ancestry, and one was associated with a glabra-fly (Figure 4.4), indicating female glabra-flies will oviposit on hybrid plants, and their offspring can survive to adulthood there. In addition, at least one coriacea-fly x glabra-fly offspring survived to adulthood on a hybrid plant, whereas most hybrid flies are found on *I. coriacea*. Based on these data, I cannot say whether the individual is the result of a glabra-fly female mating with a coriacea-fly male, but given the glabra-fly that emerged from the other hybrid plant, it is a distinct possibility. Further observations are needed to determine which flies are more likely to move among the different holly species and their hybrids, and which are more likely to oviposit on a non-natal host species.

Patterns of introgression

When introducing the hybrid bridge hypothesis, Floate and Whitham (1993) made specific predictions regarding the movement of taxa between parental plants and their hybrids: as the degree of hybridization and backcrossing to a parental species increases,

the number of intermediate steps between that parental species and hybrids should also increase, increasing the likelihood the insects will move to the hybrid plants. Therefore, if hybridization is asymmetrical between parental host species, insects should move primarily in the same direction as the asymmetry.

Asymmetrical introgression was found in the plants, with primarily *I. glabra* alleles in the *I. coriacea* genetic background (Chapter 3). There are several potential explanations for this asymmetry. First, the abundance of *I. glabra* is much larger than *I. coriacea*, both within a given location and over a larger geographic range. Although F_1 individuals are more likely to backcross to the more abundant species, backcrosses to the less abundant species will be easier to genetically discern from parental and F_1 individuals, resulting in asymmetrical introgression, with primarily alleles from the more abundant species in the genetic background of the less abundant species (Nason et al. 1992; Carney et al. 2000; Burgess et al. 2005). Because *I. glabra* is more abundant than *I. coriacea*, abundance alone could explain the higher number of individuals identified as backcrosses to *I. coriacea*.

Alleles conferring increased fitness are expected to introgress more often than neutral or deleterious alleles (Barton 2001; Borge et al. 2005; Whitney et al. 2006). *Ilex glabra* tolerates a wider range of ecological conditions than *I. coriacea* including soil texture, calcium carbonate, pH, salinity, and temperature (USDA 2012), and is also more tolerant of dry conditions (Mohlenbrock 1976; Brooks et al. 1993). If competition for space and resources is important, introgression of traits allowing greater tolerance of variation in these conditions would be more likely to allow *I. coriacea* to potentially

expand its microhabitat whereas less would be gained by *I. glabra*, matching the observed pattern in these species.

Range expansions are also expected to show patterns of unidirectional introgression from the local species into the invading species (Currat et al. 2008; Excoffier et al. 2009). If *I. glabra* has always had a more northern distribution than *I. coriacea*, and both species were pushed further south during the Pleistocene, when the species moved back north during post-glaciation, *I. coriacea* could have moved into the current range, still occupied by *I. glabra*.

As predicted by Floate and Whitham (1993), the flies show the same pattern of asymmetrical introgression consisting of primarily glabra-fly alleles in the coriacea-fly genetic background (Chapter 2). Not surprisingly, the morphology of the leaves in *I. coriacea* x *I. glabra* hybrids more closely resemble those of *I. coriacea* than *I. glabra* (Chapter 3), indicating traits important to host use by the flies could likely show the same pattern. However, the same pattern of asymmetry could also be coincidence. Flies on *I. glabra* are multivoltine whereas flies on *I. coriacea* are univoltine (Scheffer 2002). If voltinism has at least a partial environmental component linked to the host plant (Chapter 1), F_1 and backcrossed flies on *I. glabra* will have multiple generations in which they will likely mate back to the parental glabra-flies, potentially masking bidirectional gene flow by eliminating easily identifiable glabra-fly backcrosses. The development time of flies on hybrid plants remains unknown, so I cannot predict how it may differ from that on *I. coriacea* or *I. glabra*, if at all. Work is needed to determine the cause of delayed development of *P. glabricola* on *I. coriacea*, and how it may change in hybrid plants.

Previous work suggests *I. glabra* was the ancestral host for *P. glabricola*, and the flies expanded onto *I. coriacea* (Chapter 2). If a hybrid bridge was responsible for the initial host range expansion and backcrossing increases the number of intermediate steps between parental taxa, current patterns of introgression indicate the initial move from *I. glabra* to an intermediate likely required more change than from the intermediate to *I. coriacea* but less of a shift than directly from *I. glabra* to *I. coriacea* (Floate and Whitham 1993). This suggests either the initial hybrid host plants (presumably F_1) were phenotypically similar to *I. glabra* in traits that mattered to the flies or that gene flow among *I. coriacea* and *I. glabra* has changed since the initial expansion. Hybrids between *I. coriacea* and *I. glabra* contain a phenotypic mosaic of parental, intermediate, and transgressive traits (Chapter 3), suggesting multiple hybrid plants could have been colonized by glabra-flies on an evolutionary time scale. In addition, the relative abundance of the two host plant species has likely changed over time, and if abundance is responsible for asymmetrical introgression, introgression may have been more equally bidirectional in the past. Either way, it appears possible that hybrid plants may have allowed the initial host range expansion of flies in this highly specialized lineage.

Potential mechanisms

Many factors could be affecting the performance of *P. glabricola* on the hollies and their hybrids. Phytophagous insects are known to respond to the genetic composition of plants via their defensive chemistry (i.e. secondary metabolites; Bangert et al. 2006). Many holly species contain ursolic acid, phenylpropanoids, and arbutin (Choi et al. 2005), all of which have been associated with plant defense (Martin 1964; Levin 1971;

Korkina 2007); however *I. coriacea* and *I. glabra* have not yet been examined for the presence of these compounds.

A recent meta-analysis revealed physical plant traits, rather than chemical ones, have the strongest negative correlation with endophytes, followed by phenological traits and physiological traits such as water content and nitrogen concentration (Carmona et al. 2011). As apparent by their names, *I. coriacea* and *I. glabra* are both coriaceous, containing thick, leathery, and highly cutinized leaves (Caughey 1945), and glabrous, without surface ornamentation such as bristles or hairs. The waxy leaves have no effect on transpiration rates (Caughey 1945), but could potentially serve as protection against oviposition from leaf-miners (Zalucki et al. 2002).

Phenologically, *I. coriacea* blooms weeks before *I. glabra* for at least part of their overlapping range (Godfrey 1988), so the plants could also differ in the timing of new growth. Physiologically, *I. glabra* is considered less nutritious than other plants found in pocosin habitats (Smith et al. 1956), and its growth is deterred by competing plant species (Hagan et al. 2009, 2010). If nutrition content of hybrid plants differs from parental plants, it could affect host choice and survival of the flies.

Plants can also indirectly control herbivory by attracting predators and parasitoids. Parasitoids are likely the highest source of mortality in these flies, with rates varying from 50-100% parasitism among locations (JBH *unpublished*). Reduced parasitism rates on hybrid plants could allow greater survival, increasing the likelihood coriacea-flies and glabra-flies will survive and encounter one another on the hybrid plants (Fritz et al. 1999). Estimates of parasitism rates on the hybrid plants in this study (60%) appear to be intermediate between rates on *I. glabra* (64%) and those on *I. coriacea*

(52%). To fully understand how hybrid plants are serving as hybrid bridges, more work needs to be conducted to examine the nature of host plant selection by *P. glabricola*, and the specific plant traits that affect larval performance on these species and these hybrids.

I must point out that not all hybrid plants had leaf-mines (Table 4.4). I have not yet been able to determine whether flies avoid ovipositing on these plants, larvae are unable to survive, or plants drop the leaves with mines. Nonetheless, it indicates that some individual hybrid plants could serve as barriers rather than bridges. Hybrid plants likely represent a phenotypic mosaic of traits (Lexer et al. 2009; Chapter 3), where different combinations of novel, intermediate, and parental traits can be found among hybrid individuals. The particular combination of traits will be controlled by the particular combination of alleles from parental species combined with genetic recombination in backcrosses (Whitham 1989; Fritz 1999; Dungey et al. 2000). The effects of these combinations on herbivores will depend on the particular trait of interest and the composition of hybrids in that location. Having evidence for both hybrid bridges and hybrid barriers is not a paradox, but is just representative of the natural variation present in host plant populations.

The evolutionary and ecological influence of hybrid bridges and barriers will likely vary among populations. If some populations have high numbers of hybrids that serve as barriers, herbivores will likely be more adapted to parental species in those populations, and may have increased preference for parental plants to avoid the hybrid barriers (Barton 2001). On the other hand, populations with high numbers of hybrids that serve as bridges will tend have less specialized herbivores and potentially less stringent preferences. The combination of bridges and barriers among locations will likely result in

a geographic mosaic of genetic divergence among associated herbivore populations (Thompson 2005; Edelaar and Benkman 2006; Barbour et al. 2009; Thompson 2009; Marsden et al. 2011; Barman et al. 2012).

Conclusions

Variation in average adjusted hybrid indices among locations indicates a geographic mosaic of hybridization between *I. coriacea* and *I. glabra*, and a geographic mosaic of genetic divergence among host forms of *P. glabricola*. Southern populations of both host plants and the flies had higher average adjusted hybrid indices than northern populations, likely reflecting environmental variation such as differences in relative abundance of *I. glabra* among locations, which then cascaded through the plants to affect the flies.

The positive correlation between host plant hybridization and gene flow between host forms of *P. glabricola* suggest hybrid plants serve as a bridge for the flies between parental host plant species. Hybrid plants could be responsible for the initial host range expansion from *I. glabra* to *I. coriacea* in these flies. Since the initial expansion, differences in development time on each host have resulted in host plant-mediated genetic divergence among populations on each host. Subsequent adaptation to each host is driving ecological speciation among the host forms of the flies. However, the host forms are not yet new species, potentially due to gene flow promoted by hybrid bridges.

Much work on plant-insect coevolution has focused on host-range expansions followed by genetic divergence among lineages due to specialization and reproductive isolation on each host (Weintraub et al. 1995; Hawthorne and Via 2001; Ronquist and Liljeblad 2001; Nosil 2002; Janz et al. 2006; Janz and Nylin 2008). Hybrid bridges could

help explain how host range expansions in phytophagous insects first occur, particularly in highly specialized insect lineages (Kelley and Farrell 1998; Stireman 2005; Janz and Nylin 2008; Groot et al. 2011), such as *Phytomyza* (Spencer et al. 1986; Spencer 1990). Hybrids intermediate in traits important for host-use could ease the transition to the new host species (Floate and Whitham 1993). As insects adapt to the new host, genetic divergence is likely to increase due to divergent selection. Variation in hybridization rates among host plants and gene flow among insects and the genetic basis of and strength of selection on host preference and performance would likely result in a geographic mosaic of genetic divergence among host forms of insects, which if coupled with reproductive isolation, could result in a geographic mosaic of speciation (Thompson 2005; Edelaar and Benkman 2006; Barbour et al. 2009; Thompson 2009; Marsden et al. 2011; Barman et al. 2012).

Knowing hybridization is common in plants (Ellstrand et al. 1996; Rieseberg 1997), it is likely that hybrid bridges and barriers exist in many plant-insect systems, and could be largely responsible for the high diversity of species in both. The cost and effort required to generate the number and types of markers with the number of individuals needed to detect hybridization are decreasing rapidly (Glenn 2011), improving our ability to test how hybridization affects the evolution of interacting species. Increased effort will likely reveal that plant hybridization is the missing link explaining how adaptive radiations proceed in highly specialized lineages of insects.

Table 4.1: Genotyped sample sizes from each population.

			Ilex coriacea			*Ilex glabra*		
State	**Site**	**Population**	**Plants**	**Flies**	**Combo**	**Plants**	**Flies**	**Combo**
FL	Apalachicola National Forest	Hunters (HUN)	9	6	4	8	1	1
		Sopchoppy (SOP)	5	0	0	1	0	0
	Etoniah Creek State Forest	East V (EAV)	0	0	0	10	0	0
		Stuck in Sand (SIS)	10	5	4	8	9	7
GA	Crooked River State Park	Crooked River (CRG)	0	0	0	10	3	2
SC	Francis Marion National Forest	Big Ocean Bay (BOB)	18	19	7	13	21	3
		Wambaw Trail (WAM)	20	19	12	12	18	1
NC	Croatan National Forest	Catfish Lake (CAT)	10	18	0	9	3	0
		Road 152 (152)	23	27	14	10	21	1
VA	Great Dismal Swamp National Wildlife Refuge	Great Dismal Swamp (GDS)	9	2	1	9	1	0
DE	Cape Henlopen State Park	Cape Henlopen (CHE)	0	0	0	8	10	7
		Total	104	96	42	98	87	22

'Combo' refers to combinations of individual genotyped flies and the genotyped plant from which they were collected.

Table 4.2. Adjusted hybrid indices for plant populations. The standard hybrid index was adjusted so that all 'parental' individuals have an index of 0 and an index above 0 indicates some level of mixed genotype (see text). Adjusted indices were then averaged over all individuals in a population.

Population	N	Mean	Standard Deviation	Standard Error of Mean
HUN	17	0.052938	0.132544	0.032147
SOP	6	0.072731	0.143235	0.058475
EAV	10	0.014748	0.028223	0.008925
SIS	18	0.026783	0.04746	0.011187
CRG	10	0.00803	0.013211	0.004178
BOB	31	0.025078	0.085422	0.015342
WAM	32	0.020816	0.059917	0.010592
152	33	0.015237	0.049879	0.008683
CAT	19	0.026697	0.067206	0.015418
GDS	18	0.014552	0.021252	0.005009
CHE	8	0.006347	0.015464	0.005467

Table 4.3. Adjusted hybrid indices for fly populations. The standard hybrid index was adjusted so that all 'parental' individuals have an index of 0 and an index above 0 indicates some level of mixed genotype (see text). Adjusted indices were then averaged over all individuals in a population.

Population	N	Mean	Standard Deviation	Standard Error of Mean
HUN	7	0.278335	0.181328	0.068536
SIS	14	0.172567	0.164555	0.043979
CRG	3	0.121203	0.128849	0.074391
BOB	40	0.094846	0.110644	0.017494
WAM	37	0.104827	0.124812	0.020519
152	48	0.142987	0.151814	0.021912
CAT	21	0.208212	0.142443	0.031084
GDS	3	0.237584	0.244655	0.141252
CHE	10	0.065696	0.092991	0.029406

Figure 4.1: Endemic range *Ilex coriacea* and *I .glabra* with collection sites labeled.

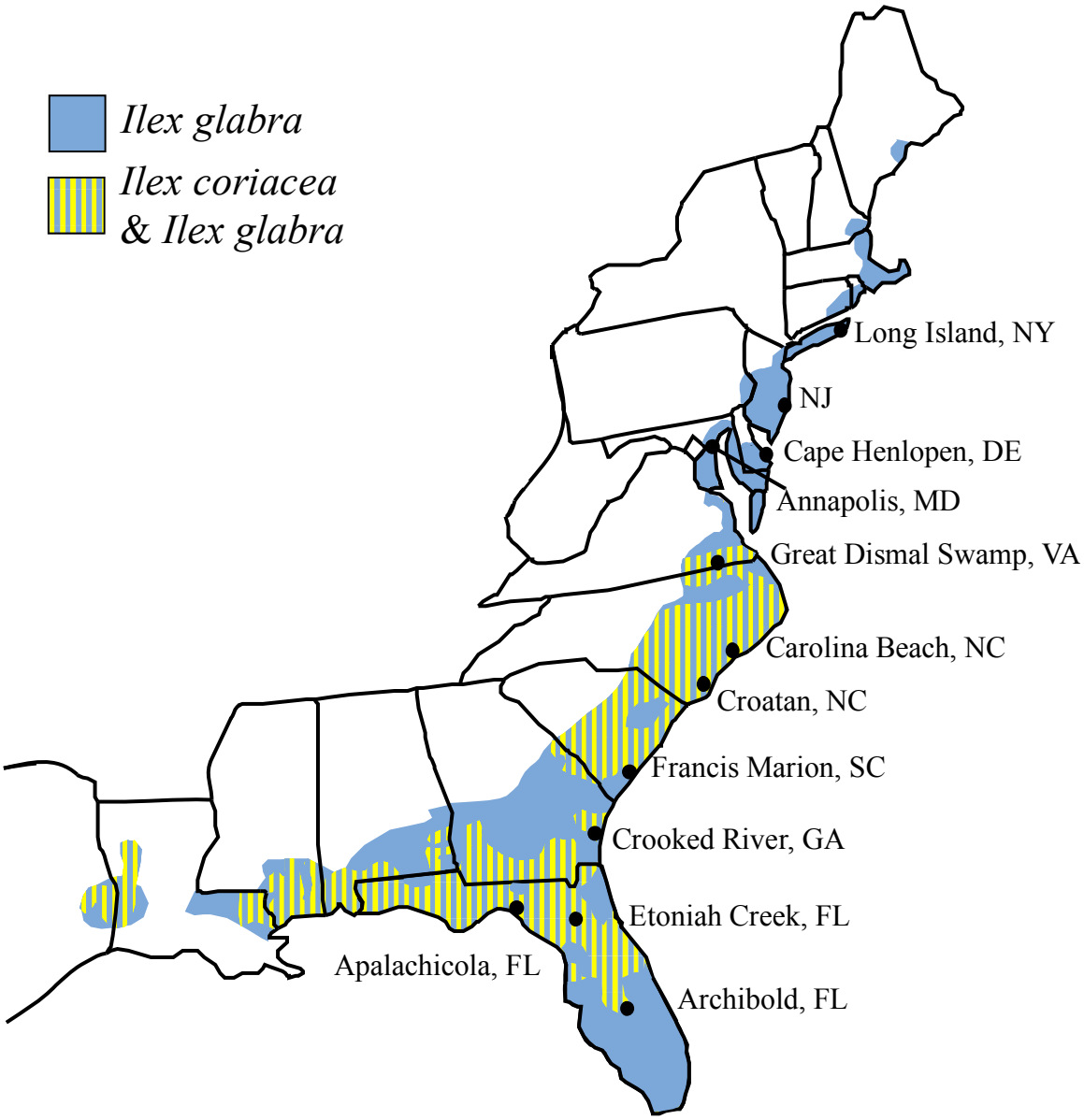


Figure 4.2. Hypothesized effects of gene flow in plants on gene flow in insects. **Ha**: If traits important for host use in insects are intermediate in hybrid plants, insects from both parental host plant species could encounter one another on hybrid plants, potentially resulting in gene flow between insects that otherwise would not encounter one another. Therefore, the more hybridization found in a given location with both host plants, the more gene flow that would be expected to be seen between host-associated insect populations or species. **Hb**: If traits important for host use in insects are novel or transgressive in hybrid plants, they could prevent insects from either parental host plant species from using the novel host, potentially selecting for greater host fidelity, decreasing gene flow between host-associated insect populations or species. **Hc**: If traits important for host use in insects display a range of phenotypes from parental to intermediate to novel, there may be no association between hybridization in host plants and gene flow in insects.

Figure 4.2

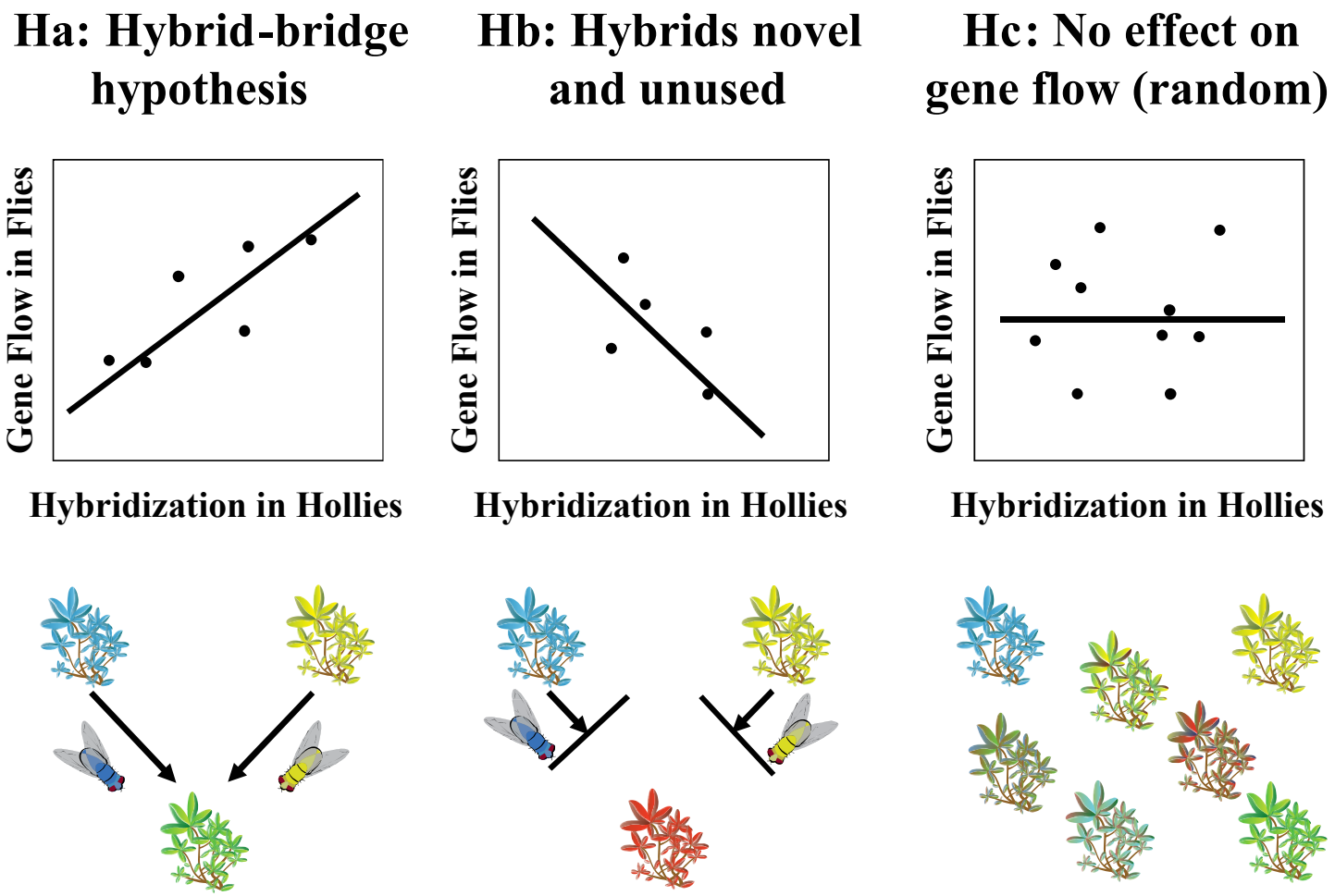

Ha: Hybrid-bridge hypothesis
Hb: Hybrids novel and unused
Hc: No effect on gene flow (random)
Gene Flow in Flies
Hybridization in Hollies
Gene Flow in Flies
Hybridization in Hollies
Gene Flow in Flies
Hybridization in Hollies

Figure 4.3. Adjusted hybrid index: The hybrid index varies between 0 and 1 where 0 represents an individual from species (or host-associated population) A with no mixed ancestry and 1 represents an individual from species (or host-associated population) B with no mixed ancestry. Individuals with hybrid indices between 0 and 1 represent individuals with some degree of mixed ancestry, where 0.5 would represent an F_1 hybrid. If hybrid indices are averaged in a location with both A and B, the average would likely be an intermediate value closer to the species (or host-associated population) with the larger sample size. Therefore, hybrid indices were standardized by subtracting any values greater than 0.5 from 1, resulting in values between 0 and 0.5 where 0 represents parental and 0.5 represents an F_1 hybrid. The image on the left contains the original hybrid indices of individuals from NC followed by their adjusted hybrid index on the right. Once values have been standardized, they can be averaged for a population to obtain a comparable estimate of the degree of hybridization within a given population.

Figure 4.3

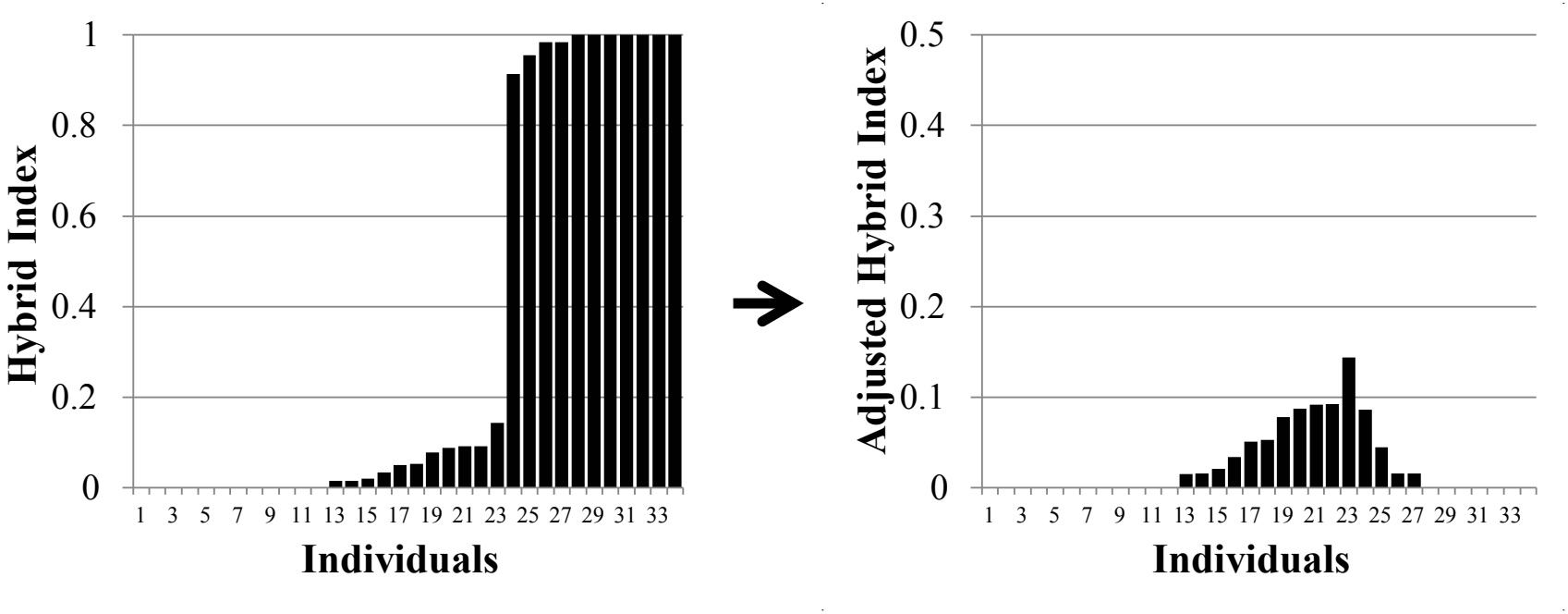

Hybrid Index
1
0.8
0.6
0.4
0.2
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33
Individuals
Adjusted Hybrid Index
0.5
0.4
0.3
0.2
0.1
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33
Individuals

Figure 4.4. Comparison of hybrid indices of individual flies on their host plants. Hybrid indices were generated based on AFLPs. Shape indicates plant status and color indicates fly status using a 10% threshold to be considered a 'hybrid'.

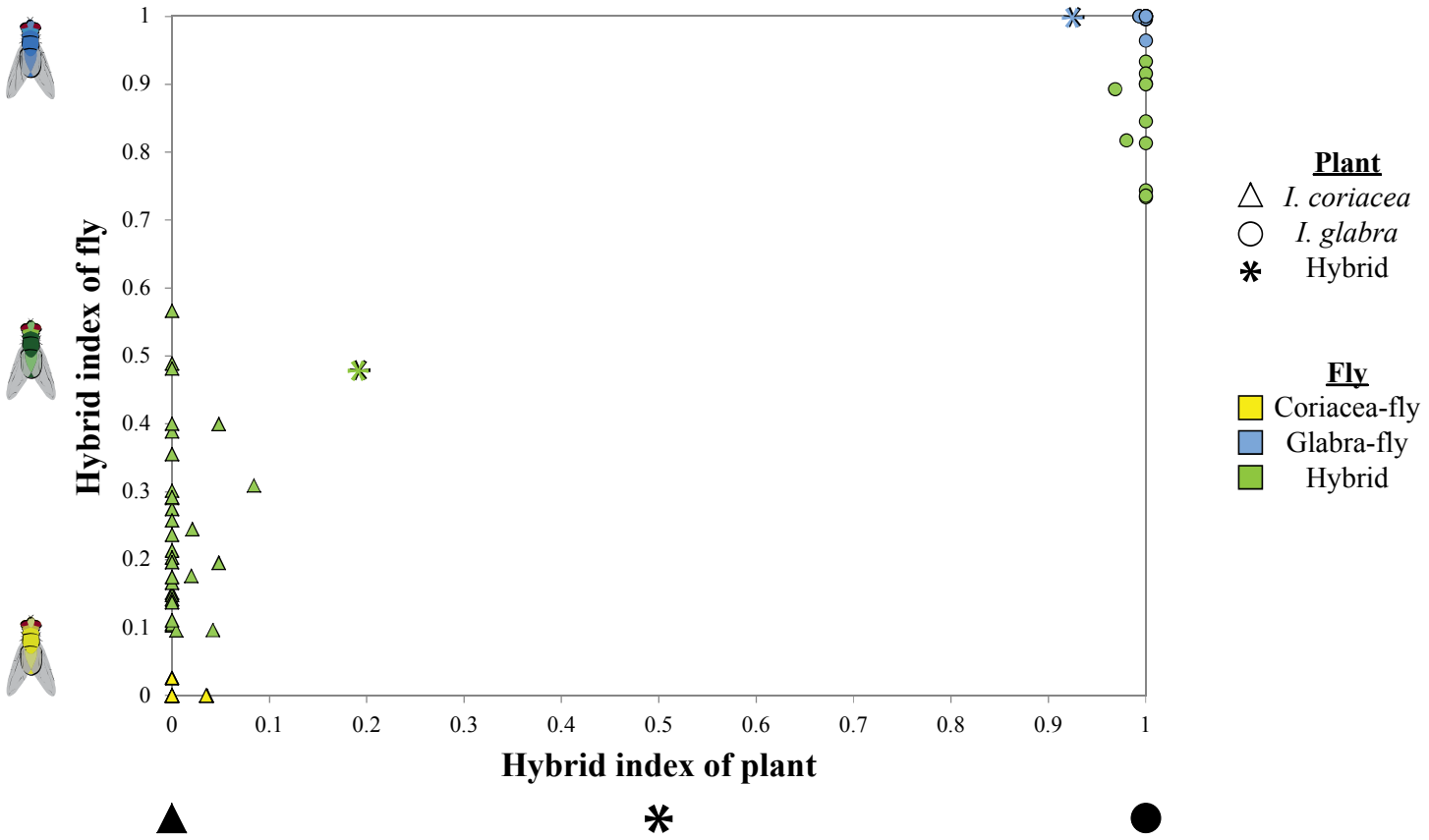
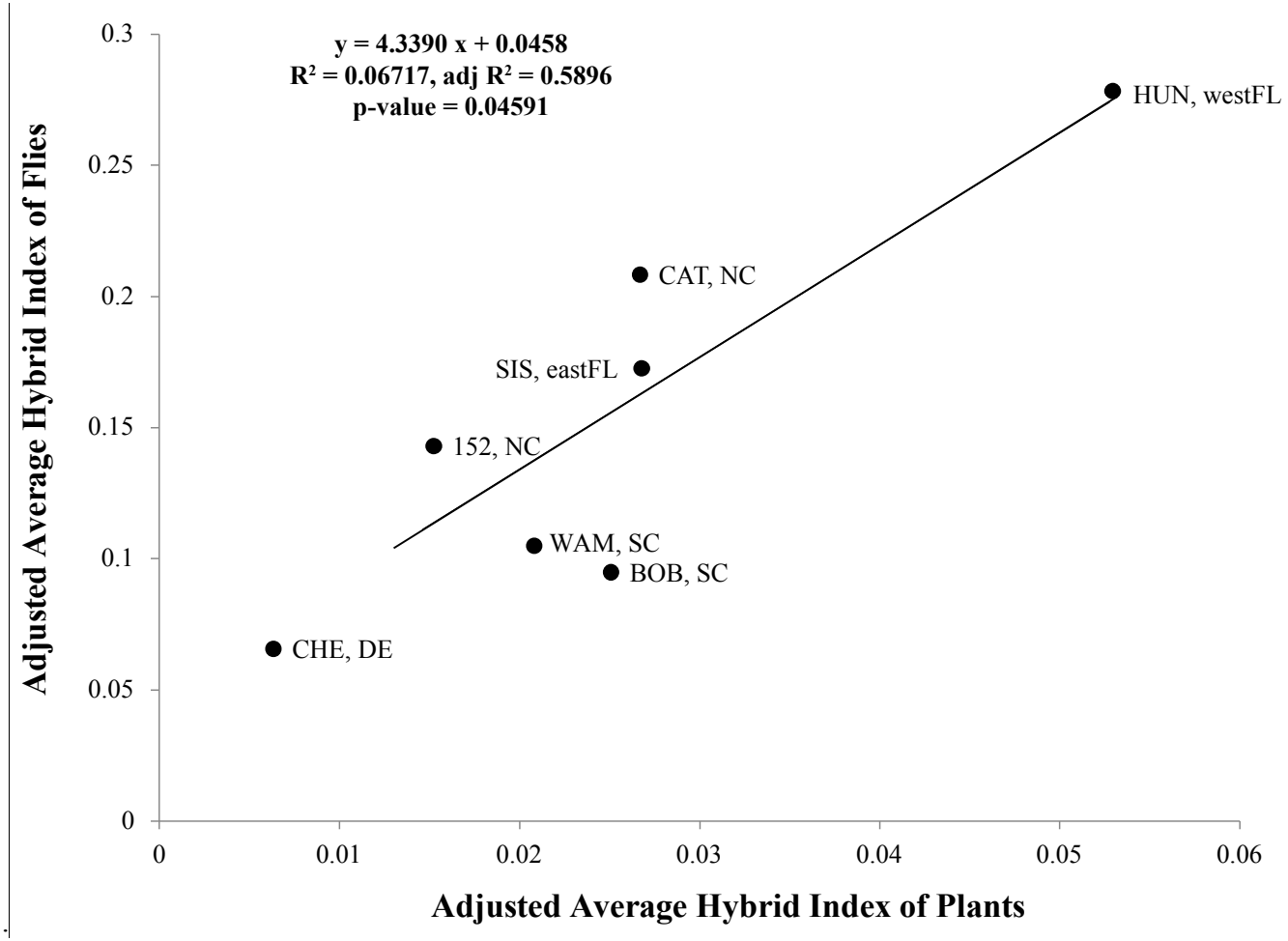


Figure 4.5. Average adjusted hybrid indices of populations. Populations had a minimum of five individuals present. Population CHE was left out of the regression analysis because it is out of the range of *I. coriacea*.



APPENDIX A: Code written in R to calculate linkage disequilibrium between dominant markers

```
#########################################################################
# Code for calculating Linkage Disequilibrium between dominant loci based on
# equations from Hill 1974.
#########################################################################

rm(list=ls())

utils:::setWindowTitle(paste("=",getwd()))

##########################      READ ME     ##############################
# The input file should be a tab-delimited text file
# The file should consist of a first column identifying a sample (individual)
# followed by the markers
# The first row should be the marker names
# The markers should all be in binary format.
# This is not set up to handle missing data, nor is it set up to handle data
# with more than 2 alleles or haplotypes.  I may be able to modify this as needed.
#########################################################################

cat("\n")                                          # outputs an empty line
cat("Enter input file name","\n")                  # Don't forget the .txt
inputfile<-readLines(n=1)

cat("Enter output file name","\n")                 # Don't forget the .txt
outputfile<-readLines(n=1)

genos<-read.delim(paste(inputfile), header=T, row.names=1)

samplenames<-row.names(genos)
markernames<-colnames(genos)

# The following sets up the table for the LD calculations

# markers 1 and 2 are each a column for a marker to be compared
# obs is the table which should be the output of the observed values

makeobserved<-function(marker, othermarker) {
  samps<-length(marker)
  marker1<-marker
  marker2<-othermarker
  obsrows<-c("A-","aa","Total")
```

```
 obscols<-c("B-","bb","Total")
 obs=matrix(data=NA, nrow=3, ncol=3)
 rownames(obs)<-obsrows
 colnames(obs)<-obscols
 AB=0
 aB=0
 Ab=0
 ab=0
 for(n in 1:samps){
  if(marker1[n]==1){
   if(marker2[n]==1) AB=AB+1
   if(marker2[n]==0) Ab=Ab+1
  }                                  ## These count the number of samples in each
  if(marker1[n]==0){                 ## combination of alleles for the two markers
   if(marker2[n]==1) aB=aB+1
   if(marker2[n]==0) ab=ab+1
  }

 }                                   # There is probably an easier way to do this
 obs[1,1]=AB                         # but I'm not shooting for clean code.
 obs[1,2]=Ab                         # Here I'm setting up the observed matrix.
 obs[2,1]=aB
 obs[2,2]=ab
 obs[1,3]=sum(obs[1,1:2])
 obs[2,3]=sum(obs[2,1:2])
 obs[3,1]=sum(obs[1:2,1])
 obs[3,2]=sum(obs[1:2,2])
 obs[3,3]=sum(obs[3,1:2])
 return(obs)
}

makep<-function(obs) {   # obs is the table of observed values
 obss<-obs
 sumaa<-obss[2,3]
 tot<-obss[3,3]
 pval=1-sqrt((sumaa/tot))
 return(pval)
}

makeq<-function(obs) {
 obss<-obs
 sumbb<-obss[3,2]
 tot<-obss[3,3]
 qval=1-sqrt((sumbb/tot))
 return(qval)
}
```

```
makef22<-function(obs) {
 obss<-obs
 aabb<-obss[2,2]
 tot<-obs[3,3]
 f22=sqrt(aabb/tot)
 return(f22)
}

makeD<-function(obs) {
 obss<-obs
 f22<-makef22(obss)
 sumaa<-obss[2,3]
 sumbb<-obss[3,2]
 tot<-obss[3,3]
 LD=f22-sqrt((sumaa*sumbb))/tot
 return(LD)
}

makeK<-function(obs) {
 obss<-obs
 LD<-makeD(obss)
 pval<-makep(obss)
 if(pval==0) {      # removing NAs
  pval<-0.000001
 }
 qval<-makeq(obss)
 if(qval==0) {              # removing NAs
  qval<-0.000001
 }
 tot<-obss[3,3]
 kval=(4*tot*(LD^2))/(pval*(2-pval)*qval*(2-qval))
 return(kval)
}

# for eventually outputting the calculated values

fullLD=matrix(data=NA, nrow=1, ncol=11)
colnames(fullLD)<-c("Compare","Marker1", "Marker2", "A_B_", "A_bb", "aaB_",
"aabb", "p", "q", "D", "k")

#samplenames, markernames, genos

nummark<-length(markernames)

for(i in 1:(nummark-1)){
```

```
  print("i=")
  print(i)
  remaining<-i+1                          # the for statements will go through every
  for(j in remaining:nummark){            # combination of markers
    marker1<-genos[,i]
    marker2<-genos[,j]
    observed<-makeobserved(marker1, marker2)
    AB<-observed[1,1]
    Ab<-observed[1,2]
    aB<-observed[2,1]
    ab<-observed[2,2]
    pval<-makep(observed)
    qval<-makeq(observed)
    Dval<-makeD(observed)
    Kval<-makeK(observed)
    comparename=paste(markernames[i],markernames[j],sep="-")
    fullLDentry<-c(comparename,markernames[i],markernames[j], AB, Ab, aB, ab, pval,
qval, Dval, Kval)
    fullLD<-rbind(fullLD, fullLDentry)
  }
}
rownames(fullLD)<-fullLD[,1]
fullLD<-fullLD[2:nrow(fullLD),2:ncol(fullLD)]
fullLD<-as.data.frame(fullLD)
likeli<-as.numeric(as.vector(fullLD$k))
pvalue<-vector(mode="numeric", length=nrow(fullLD))
for(i in 1:nrow(fullLD)){
  if(!is.na(likeli[i])){
    pvalue[i]=pchisq(likeli[i], df=1, lower.tail=F)
  }
}
fullLD<-cbind(fullLD, pvalue)

write.table(fullLD, file=outputfile, quote=F, sep="\t", append=F)
```

APPENDIX B: Summary Data for *Phytomyza glabricola*

Sample	Host	Sex	Population	Location	Collection	AFLP	EF-1alpha
152C004	C	F	152	NC	2006	X	X
152C013	C	M	152	NC	2006	X	X
152C026	C	M	152	NC	2006	X	X
152C031	C	M	152	NC	2006	X	X
152C032	C	F	152	NC	2006	X	X
152C039	C	F	152	NC	2006	X	X
152C042	C	M	152	NC	2006	X	X
152C059	C	F	152	NC	2006	X	X
152C061	C	M	152	NC	2006	X	X
152C062	C	F	152	NC	2006	X	X
152C077	C	M	152	NC	2006	X	X
152C092	C	F	152	NC	2006	X	X
152C096	C	F	152	NC	2006	X	X
152C099	C	M	152	NC	2006		X
152C101	C	F	152	NC	2006		X
152C102	C	M	152	NC	2006	X	X
152C123	C	F	152	NC	2006	X	X
152C127	C	F	152	NC	2006	X	X
152C130	C	M	152	NC	2006	X	X
152C142	C	M	152	NC	2006	X	X
152C143	C	F	152	NC	2006	X	X
152C190	C	F	152	NC	2006	X	X
152C223	C	M	152	NC	2007	X	X
152C234	C	M	152	NC	2007		X
152C248	C	F	152	NC	2007	X	X
152C258	C	F	152	NC	2007	X	
152C264	C	F	152	NC	2007	X	X
152C265	C	?	152	NC	2007		X
152C271	C	F	152	NC	2007	X	X
152C272	C	F	152	NC	2007		X
152C273	C	M	152	NC	2007	X	X
152C280	C	M	152	NC	2007		X
152C288	C	M	152	NC	2007	X	X
152G001	G	M	152	NC	2006	X	X
152G002	G	F	152	NC	2006	X	X
152G012	G	M	152	NC	2006	X	X
152G013	G	M	152	NC	2006		X
152G015	G	M	152	NC	2006	X	X
152G018	G	F	152	NC	2006	X	X
152G030	G	M	152	NC	2006		X

Sample	Host	Sex	Population	Location	Collection	AFLP	EF-1alpha
152G033	G	F	152	NC	2006		X
152G034	G	M	152	NC	2006	X	X
152G035	G	F	152	NC	2006	X	X
152G037	G	F	152	NC	2006	X	X
152G038	G	M	152	NC	2006	X	X
152G040	G	F	152	NC	2006	X	X
152G053	G	M	152	NC	2006		X
152G066	G	M	152	NC	2006	X	X
152G068	G	F	152	NC	2006	X	X
152G075	G	F	152	NC	2006	X	X
152G086	G	F	152	NC	2006	X	X
152G093	G	M	152	NC	2006	X	X
152G096	G	F	152	NC	2006	X	X
152G098	G	M	152	NC	2006	X	X
152G109	G	F	152	NC	2006	X	X
152G116	G	F	152	NC	2006	X	X
152G123	G	F	152	NC	2006		X
152G126	G	M	152	NC	2006		X
152G164	G	M	152	NC	2007	X	X
152G167	G	F	152	NC	2007		X
152G183	G	M	152	NC	2007		X
152G199	G	M	152	NC	2007	X	X
BOBC006	C	M	BOB	SC	2006	X	X
BOBC007	C	F	BOB	SC	2006	X	X
BOBC012	C	F	BOB	SC	2006	X	
BOBC019	C	F	BOB	SC	2006	X	
BOBC023	C	F	BOB	SC	2006	X	X
BOBC037	C	F	BOB	SC	2006	X	X
BOBC039	C	F	BOB	SC	2006	X	X
BOBC046	C	M	BOB	SC	2006	X	X
BOBC047	C	M	BOB	SC	2006		X
BOBC049	C	F	BOB	SC	2006	X	X
BOBC050	C	M	BOB	SC	2006		X
BOBC074	C	F	BOB	SC	2006		X
BOBC076	C	M	BOB	SC	2006	X	X
BOBC084	C	F	BOB	SC	2006	X	X
BOBC127	C	M	BOB	SC	2006	X	X
BOBC128	C	F	BOB	SC	2006	X	X
BOBC130	C	F	BOB	SC	2006	X	X
BOBC134	C	M	BOB	SC	2006		X
BOBC149	C	F	BOB	SC	2006	X	X
BOBC196	C	M	BOB	SC	2007	X	X

Sample	Host	Sex	Population	Location	Collection	AFLP	EF-1alpha
BOBC198	C	M	BOB	SC	2007	X	X
BOBC228	C	F	BOB	SC	2007		X
BOBC230	C	M	BOB	SC	2007	X	X
BOBC241	C	F	BOB	SC	2007		X
BOBC243	C	F	BOB	SC	2007	X	
BOBG001	G	M	BOB	SC	2006	X	X
BOBG002	G	M	BOB	SC	2006	X	X
BOBG003	G	M	BOB	SC	2006	X	X
BOBG004	C	F	BOB	SC	2006		X
BOBG005	G	M	BOB	SC	2006	X	X
BOBG007	G	M	BOB	SC	2006	X	X
BOBG010	G	M	BOB	SC	2006	X	X
BOBG034	G	M	BOB	SC	2006	X	X
BOBG045	G	F	BOB	SC	2006	X	X
BOBG057	G	M	BOB	SC	2006	X	X
BOBG067	C	M	BOB	SC	2006		X
BOBG090	G	F	BOB	SC	2006	X	X
BOBG094	G	F	BOB	SC	2006	X	X
BOBG095	G	F	BOB	SC	2006	X	X
BOBG104	G	F	BOB	SC	2006	X	X
BOBG111	G	F	BOB	SC	2006	X	
BOBG114	G	F	BOB	SC	2006	X	X
BOBG120	G	F	BOB	SC	2006	X	X
BOBG128	G	M	BOB	SC	2006	X	X
BOBG158	G	?	BOB	SC	2007	X	X
BOBG159	G	?	BOB	SC	2007		X
BOBG169	G	F	BOB	SC	2007	X	X
BOBG174	G	F	BOB	SC	2007	X	X
BOBG190	G	M	BOB	SC	2007		X
BOBG198	G	M	BOB	SC	2007	X	X
CATC004	C	F	CAT	NC	2006	X	X
CATC010	C	M	CAT	NC	2006	X	X
CATC049	C	F	CAT	NC	2006	X	X
CATC051	C	F	CAT	NC	2006	X	X
CATC076	C	F	CAT	NC	2006		X
CATC105	C	F	CAT	NC	2006	X	X
CATC113	C	M	CAT	NC	2006		X
CATC115	C	M	CAT	NC	2006	X	X
CATC119	C	M	CAT	NC	2006	X	X
CATC124	C	M	CAT	NC	2006	X	X
CATC135	C	M	CAT	NC	2006	X	X
CATC145	C	M	CAT	NC	2006	X	X

Sample	Host	Sex	Population	Location	Collection	AFLP	EF-1alpha
CATC148	C	F	CAT	NC	2006		X
CATC158	C	M	CAT	NC	2006	X	X
CATC159	C	M	CAT	NC	2006	X	X
CATC168	C	M	CAT	NC	2006	X	X
CATC172	C	M	CAT	NC	2006	X	X
CATC175	C	M	CAT	NC	2006		X
CATC176	C	F	CAT	NC	2006	X	X
CATC179	C	M	CAT	NC	2006	X	X
CATC183	C	F	CAT	NC	2006	X	X
CATC189	C	M	CAT	NC	2006	X	X
CATG001	G	F	CAT	NC	2006		X
CATG013	G	F	CAT	NC	2006	X	X
CATG025	G	M	CAT	NC	2006		X
CATG038	G	F	CAT	NC	2006	X	X
CATG073	G	F	CAT	NC	2006	X	X
CHEG005	G	?	CHE	DE	2007		X
CHEG033	G	M	CHE	DE	2007	X	X
CHEG048	G	F	CHE	DE	2007	X	X
CHEG049	G	M	CHE	DE	2007	X	X
CHEG059	G	?	CHE	DE	2007		X
CHEG064	G	?	CHE	DE	2007		X
CHEG088	G	F	CHE	DE	2007	X	X
CHEG095	G	F	CHE	DE	2007	X	X
CHEG096	G	F	CHE	DE	2007	X	X
CHEG106	G	F	CHE	DE	2007		X
CHEG107	G	M	CHE	DE	2007	X	X
CHEG108	G	F	CHE	DE	2007		X
CHEG109	G	?	CHE	DE	2007	X	X
CHEG114	G	M	CHE	DE	2007	X	X
CHEG122	G	F	CHE	DE	2007	X	X
CRGG002	G	M	CRG	GA	2007	X	X
CRGG007	G	F	CRG	GA	2007		X
CRGG008	G	F	CRG	GA	2007	X	X
CRGG014	G	larva	CRG	GA	2007	X	X
EAVG001	G	larva	EAV	EAST-FL	2007		X
EAVG002	G	larva	EAV	EAST-FL	2007		X
GDSC005	C	M	GDS	VA	2007		X
GDSC039	C	F	GDS	VA	2007		X
GDSC056	C	M	GDS	VA	2007	X	X
GDSC065	C	F	GDS	VA	2007	X	X
GDSG012	G	M	GDS	VA	2007	X	X
HUNC002	C	F	HUN	WEST-FL	2007	X	X

Sample	Host	Sex	Population	Location	Collection	AFLP	EF-1alpha
HUNC003	C	F	HUN	WEST-FL	2007	X	X
HUNC006	C	M	HUN	WEST-FL	2007	X	X
HUNC007	C	F	HUN	WEST-FL	2007	X	X
HUNC009	C	M	HUN	WEST-FL	2007	X	X
HUNC014	C	M	HUN	WEST-FL	2007	X	X
HUNG002	G	M	HUN	WEST-FL	2007	X	X
PCo15	C	?	BOB	SC	Scheffer & Hawthorne		X
PCo16	C	?	BOB	SC	Scheffer & Hawthorne		X
PCo18	C	?	BOB	SC	Scheffer & Hawthorne		X
PCo19	C	?	BOB	SC	Scheffer & Hawthorne		X
PCo21	C	?	BOB	SC	Scheffer & Hawthorne		X
PCo23	C	?	BOB	SC	Scheffer & Hawthorne		X
PCo26	C	?	BOB	SC	Scheffer & Hawthorne		X
PCo27	C	?	BOB	SC	Scheffer & Hawthorne		X
PCo28	C	?	BOB	SC	Scheffer & Hawthorne		X
PCo29	C	?	BOB	SC	Scheffer & Hawthorne		X
PCo30	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo31	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo33	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo34	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo35	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo36	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo37	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo38	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo39	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo40	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo41	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo42	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo43	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo44	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PCo45	C	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PGl14	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl15	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl16	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl17	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl18	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl20	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl21	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl22	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl23	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl24	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl25	G	?	BOB	SC	Scheffer & Hawthorne		X

Sample	Host	Sex	Population	Location	Collection	AFLP	EF-1alpha
PGl26	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl27	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl28	G	?	BOB	SC	Scheffer & Hawthorne		X
PGl31	G	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PGl39	G	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PGl40	G	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PGl41	G	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PGl42	G	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PGl44	G	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PGl45	G	?	Carolina Beach	NC	Scheffer & Hawthorne		X
PGl46	G	?	Archibold	SOUTH-FL	Scheffer & Hawthorne		X
PGl47	G	?	Archibold	SOUTH-FL	Scheffer & Hawthorne		X
PGl48	G	?	Archibold	SOUTH-FL	Scheffer & Hawthorne		X
PGl49	G	?	Archibold	SOUTH-FL	Scheffer & Hawthorne		X
PGl50	G	?	Archibold	SOUTH-FL	Scheffer & Hawthorne		X
PGl51	G	?	Archibold	SOUTH-FL	Scheffer & Hawthorne		X
PGl52	G	?	Archibold	SOUTH-FL	Scheffer & Hawthorne		X
PGl53	G	?	Archibold	SOUTH-FL	Scheffer & Hawthorne		X
PGl54	G	?	Long Island	NY	Scheffer & Hawthorne		X
PGl55	G	?	Long Island	NY	Scheffer & Hawthorne		X
PGl56	G	?	Annapolis	MD	Scheffer & Hawthorne		X
PGl62	G	?	Annapolis	MD	Scheffer & Hawthorne		X
PGl63	G	?	NJ	NJ	Scheffer & Hawthorne		X
PGl64	G	?	NJ	NJ	Scheffer & Hawthorne		X
SISC004	C	F	SIS	EAST-FL	2007	X	X
SISC014	C	M	SIS	EAST-FL	2007	X	X
SISC025	C	larva	SIS	EAST-FL	2007		X
SISC030	C	M	SIS	EAST-FL	2007	X	X
SISC040	C	M	SIS	EAST-FL	2007	X	X
SISC041	C	larva	SIS	EAST-FL	2007		X
SISC042	C	F	SIS	EAST-FL	2007	X	X
SISG003	G	M	SIS	EAST-FL	2007	X	X
SISG007	G	larva	SIS	EAST-FL	2007		X
SISG011	G	F	SIS	EAST-FL	2007	X	X
SISG032	G	M	SIS	EAST-FL	2007	X	X
SISG048	G	M	SIS	EAST-FL	2007	X	X
SISG050	G	F	SIS	EAST-FL	2007	X	X
SISG054	G	M	SIS	EAST-FL	2007		X
SISG066	G	M	SIS	EAST-FL	2007	X	X
SISG067	G	M	SIS	EAST-FL	2007	X	X
SISG069	G	M	SIS	EAST-FL	2007	X	X
SISG076	G	M	SIS	EAST-FL	2007	X	X

Sample	Host	Sex	Population	Location	Collection	AFLP	EF-1alpha
WAMC002	C	M	WAM	SC	2006	X	X
WAMC004	C	M	WAM	SC	2006	X	X
WAMC014	C	M	WAM	SC	2006	X	X
WAMC028	C	M	WAM	SC	2006		X
WAMC031	C	F	WAM	SC	2006	X	X
WAMC034	C	F	WAM	SC	2006		X
WAMC036	C	M	WAM	SC	2006	X	X
WAMC040	C	F	WAM	SC	2006	X	X
WAMC041	C	F	WAM	SC	2006		X
WAMC044	C	F	WAM	SC	2006		X
WAMC054	C	F	WAM	SC	2006	X	X
WAMC057	C	M	WAM	SC	2006	X	X
WAMC063	C	F	WAM	SC	2006	X	X
WAMC066	C	F	WAM	SC	2006		X
WAMC078	C	F	WAM	SC	2006		X
WAMC082	C	M	WAM	SC	2006	X	X
WAMC084	C	M	WAM	SC	2006	X	X
WAMC090	C	M	WAM	SC	2006		X
WAMC092	C	M	WAM	SC	2006	X	X
WAMC102	C	M	WAM	SC	2007		X
WAMC103	C	F	WAM	SC	2007	X	X
WAMC106	C	M	WAM	SC	2007		X
WAMC114	C	F	WAM	SC	2007	X	X
WAMC121	C	F	WAM	SC	2007	X	X
WAMC124	C	larva	WAM	SC	2007		X
WAMC127	C	F	WAM	SC	2007	X	
WAMC128	C	F	WAM	SC	2007	X	X
WAMC141	C	?	WAM	SC	2007	X	X
WAMC146	C	larva	WAM	SC	2007		X
WAMC148	C	F	WAM	SC	2007	X	X
WAMG001	G	M	WAM	SC	2006	X	X
WAMG005	G	M	WAM	SC	2006	X	X
WAMG008	G	M	WAM	SC	2006	X	X
WAMG012	G	F	WAM	SC	2006	X	X
WAMG016	G	F	WAM	SC	2006	X	X
WAMG020	G	M	WAM	SC	2006	X	X
WAMG031	G	F	WAM	SC	2006	X	X
WAMG037	G	F	WAM	SC	2006	X	X
WAMG038	G	F	WAM	SC	2006	X	X
WAMG040	G	M	WAM	SC	2006	X	X
WAMG043	G	M	WAM	SC	2006	X	X
WAMG050	G	F	WAM	SC	2006	X	X

Sample	Host	Sex	Population	Location	Collection	AFLP	EF-1alpha
WAMG052	G	F	WAM	SC	2006		X
WAMG055	G	F	WAM	SC	2006	X	X
WAMG062	G	F	WAM	SC	2006	X	X
WAMG067	G	M	WAM	SC	2006		X
WAMG068	G	F	WAM	SC	2006	X	X
WAMG069	G	M	WAM	SC	2006		X
WAMG073	G	M	WAM	SC	2006		X
WAMG075	G	F	WAM	SC	2006	X	X
WAMG076	G	F	WAM	SC	2006		X
WAMG092	G	M	WAM	SC	2007	X	X
WAMG096	G	M	WAM	SC	2007	X	

Hosts are host plants *Ilex coriacea* (C) and *I. glabra* (G). Sex are sex of the flies: female (F), male (M), larva, or unknown (?). Individuals sampled in 2006 and 2007 are from Cape Henlopen (CHE), Great Dismal Swamp (GDS), Croatan (152 and CAT), Francis Marion (BOB and WAM), Crooked River (CRG), Etoniah Creek (SIS), and Apalachicola (HUN) (see Figure 2.1 for map). Details for individuals not collected in 2006 or 2007 can be found in Scheffer and Hawthorne (2007).

APPENDIX C: Results of NEWHYBRIDS in *Phytomyza glabricola*

Highlighted posterior probabilities represent the different cutoffs for introgression: **backcross** in bold, ***less than 75% posterior probability of belonging to a parental type*** in bold italics, and *less than 90% posterior probability of belonging to a parental type* in italics.

Sample	Coriacea-fly	Backcross-coriacea	F1	Backcross-glabra	Glabra-fly
152C004	***0.69483***	0.30506	0.00008	0.00003	0
152C013	0.99535	0.00462	0.00002	0.00002	0
152C026	0.12764	**0.84579**	0.02624	0.00032	0
152C031	0.1273	**0.85883**	0.01384	0.00004	0
152C032	*0.8078*	0.19199	0.0002	0	0
152C039	0.97639	0.02357	0.00003	0.00001	0
152C042	0.97766	0.02227	0.00003	0.00003	0
152C059	0.99719	0.00278	0.00002	0.00001	0
152C061	0.98565	0.0143	0.00004	0	0
152C062	0.99673	0.00325	0.00002	0.00001	0
152C077	*0.75451*	0.24512	0.00033	0.00004	0
152C092	0.97206	0.0279	0	0.00003	0
152C096	0.98758	0.01239	0.00002	0.00002	0
152C102	0.02938	**0.92371**	0.04481	0.0021	0
152C123	0.98965	0.01032	0.00002	0.00001	0
152C127	***0.6564***	0.34341	0.00018	0.00001	0
152C130	0.98347	0.01648	0.00003	0.00002	0
152C142	*0.88241*	0.11747	0.00008	0.00004	0
152C143	0.99751	0.00247	0.00002	0.00001	0
152C190	0.9926	0.00736	0.00003	0.00001	0
152C223	0.96684	0.03309	0.00003	0.00004	0
152C248	0.99956	0.00042	0.00001	0	0
152C258	*0.83909*	0.16073	0.00017	0	0
152C264	*0.80683*	0.19308	0.00008	0.00001	0
152C271	0.97316	0.0268	0.00003	0	0
152C273	0.984	0.01596	0.00002	0.00002	0
152C288	0.09805	**0.86949**	0.03188	0.00058	0
152G001	0	0.00022	0	0.00116	0.99853
152G002	0	0.00001	0.00144	0.24645	*0.75211*
152G012	0	0.00021	0.00056	0.05715	0.94208
152G015	0	0	0	0.00064	0.99935
152G018	0	0	0.00001	0.00263	0.99736
152G034	0	0.00036	0.00392	0.14465	*0.85106*
152G035	0	0.00001	0.00087	0.17271	*0.82641*
152G037	0	0	0.00001	0.00173	0.99826
152G038	0	0	0	0.00329	0.99671

Sample	Coriacea-fly	Backcross-coriacea	F1	Backcross-glabra	Glabra-fly
152G040	0	0	0	0.00627	0.99372
152G066	0	0	0	0.00122	0.99878
152G068	0	0.00016	0.00249	0.14035	*0.857*
152G075	0	0	0	0.00205	0.99795
152G086	0	0	0.00021	0.03859	0.96119
152G093	0	0	0	0.00017	0.99982
152G096	0	0.00018	0.00004	0.00032	0.99947
152G098	0	0	0.00001	0.00947	0.99053
152G109	0	0	0.00001	0.00471	0.99528
152G116	0	0	0.00003	0.01343	0.98654
152G164	0	0	0	0.00034	0.99966
152G199	0	0	0	0.00012	0.99988
BOBC006	0.98802	0.01192	0.00003	0.00003	0
BOBC007	0.99334	0.00663	0.00002	0	0
BOBC012	0.9989	0.00107	0.00002	0.00001	0
BOBC019	0.99545	0.00452	0.00002	0	0
BOBC023	0.99792	0.00206	0.00002	0	0
BOBC037	0.99521	0.00478	0.00001	0	0
BOBC039	0.99689	0.00308	0.00002	0.00001	0
BOBC046	0.99641	0.00354	0.00002	0.00003	0
BOBC049	0.99987	0.0001	0.00002	0	0
BOBC076	*0.75172*	0.24811	0.00014	0.00003	0
BOBC084	0.99536	0.0046	0.00002	0.00001	0
BOBC127	0.99026	0.0097	0.00002	0.00003	0
BOBC128	0.98369	0.01628	0.00002	0.00002	0
BOBC130	0.9978	0.00217	0.00002	0.00001	0
BOBC149	0.92269	0.07724	0.00004	0.00003	0
BOBC196	*0.77289*	0.22644	0.00062	0.00005	0
BOBC198	0.98163	0.01832	0.00005	0.00001	0
BOBC230	0.98467	0.01527	0.00001	0.00004	0
BOBC243	0.98683	0.01313	0.00001	0.00002	0
BOBG001	0	0	0	0.00017	0.99982
BOBG002	0	0	0.00002	0.00862	0.99136
BOBG003	0	0	0.00001	0.00615	0.99383
BOBG005	0	0.00006	0.00005	0.00075	0.99914
BOBG007	0	0	0.0005	0.0745	0.92499
BOBG010	0	0	0	0.00023	0.99977
BOBG034	0	0	0	0.00022	0.99977
BOBG045	0	0	0.00001	0.02353	0.97645
BOBG057	0	0.00001	0.00011	0.03298	0.9669
BOBG090	0	0.00086	0.0214	0.45957	***0.51817***
BOBG094	0	0	0.00004	0.01593	0.98403

Sample	Coriacea-fly	Backcross-coriacea	F1	Backcross-glabra	Glabra-fly
BOBG095	0	0	0.00002	0.01182	0.98816
BOBG104	0	0	0.00002	0.01086	0.98911
BOBG111	0	0	0.00004	0.0102	0.98976
BOBG114	0	0.00004	0.00051	0.04628	0.95317
BOBG120	0	0	0	0.00097	0.99903
BOBG128	0	0	0	0.00133	0.99867
BOBG158	0	0	0.00016	0.08061	0.91923
BOBG169	0	0	0.00002	0.01248	0.9875
BOBG174	0	0	0.00002	0.00132	0.99866
BOBG198	0	0	0	0.00226	0.99773
CATC004	0.99275	0.00722	0.00002	0.00001	0
CATC010	*0.82866*	0.17081	0.00052	0.00001	0
CATC049	0.99732	0.00265	0.00002	0.00001	0
CATC051	0.98409	0.01588	0.00002	0.00002	0
CATC105	0.99598	0.00399	0.00002	0.00001	0
CATC115	0.91022	0.08967	0.00008	0.00004	0
CATC119	0.45149	**0.53159**	0.01686	0.00006	0
CATC124	0.9354	0.0645	0.00008	0.00002	0
CATC135	***0.6342***	0.36477	0.001	0.00003	0
CATC145	***0.74724***	0.25252	0.00022	0.00002	0
CATC158	*0.84137*	0.15844	0.00015	0.00004	0
CATC159	*0.82206*	0.17776	0.00017	0.00002	0
CATC168	*0.77915*	0.22045	0.00038	0.00003	0
CATC172	0.02232	**0.86638**	0.0754	0.03589	0
CATC176	0.98656	0.01341	0.00001	0.00002	0
CATC179	0.07718	**0.91764**	0.00509	0.00009	0
CATC183	0.99745	0.00253	0.00002	0.00001	0
CATC189	0.99546	0.00449	0.00001	0.00004	0
CATG013	0	0	0.00006	0.02188	0.97806
CATG038	0	0	0.00004	0.03677	0.96319
CATG073	0	0	0.0003	0.06826	0.93143
CHEG033	0	0	0	0.00205	0.99795
CHEG048	0	0	0	0.00114	0.99885
CHEG049	0	0	0	0.00024	0.99976
CHEG088	0	0	0.00001	0.0002	0.99979
CHEG095	0	0	0.00011	0.03809	0.96181
CHEG096	0	0	0.00002	0.01153	0.98845
CHEG107	0	0	0	0.0011	0.99889
CHEG109	0	0	0	0.00495	0.99505
CHEG114	0	0	0.0001	0.03494	0.96496
CHEG122	0	0	0.00001	0.00294	0.99705
CRGG002	0	0	0.00001	0.00247	0.99753

Sample	Coriacea-fly	Backcross-coriacea	F1	Backcross-glabra	Glabra-fly
CRGG008	0	0.00001	0.00213	0.18799	*0.80988*
CRGG014	0	0	0.00001	0.00616	0.99384
GDSC056	0.00921	**0.85928**	0.12452	0.00699	0
GDSC065	0.3398	**0.65794**	0.00225	0.00001	0
GDSG012	0	0	0	0.0034	0.9966
HUNC002	0.97846	0.0215	0.00002	0.00001	0
HUNC003	0.95707	0.04289	0.00003	0.00001	0
HUNC006	*0.89035*	0.10953	0.00009	0.00003	0
HUNC007	*0.75733*	0.24256	0.00009	0.00002	0
HUNC009	0.06793	**0.91389**	0.01777	0.00042	0
HUNC014	0.03545	**0.87577**	0.08584	0.00294	0
HUNG002	0	0	0.00004	0.02079	0.97917
SISC004	0.00633	**0.98404**	0.00924	0.00039	0
SISC014	***0.62245***	0.37695	0.00054	0.00005	0
SISC030	0.21185	**0.78772**	0.00036	0.00007	0
SISC040	0.14824	**0.79494**	0.04818	0.00863	0.00001
SISC042	0.05308	**0.94497**	0.00194	0	0
SISG003	0	0	0	0.00556	0.99443
SISG011	0	0	0.00012	0.02969	0.97019
SISG032	0	0	0	0.00028	0.99972
SISG048	0	0	0	0.01391	0.98609
SISG050	0	0.0353	0.13903	**0.60432**	0.22135
SISG066	0	0	0	0.00075	0.99925
SISG067	0	0	0	0.00096	0.99904
SISG069	0	0	0	0.00351	0.99649
SISG076	0	0	0.00009	0.01654	0.98336
WAMC002	0.16851	**0.82209**	0.00929	0.00011	0
WAMC004	0.9963	0.00364	0.00001	0.00004	0
WAMC014	0.95987	0.04005	0.00005	0.00003	0
WAMC031	0.97133	0.02863	0.00002	0.00001	0
WAMC036	0.97295	0.02699	0.00003	0.00003	0
WAMC040	0.99281	0.00715	0.00003	0.00001	0
WAMC054	***0.59293***	0.40519	0.00186	0.00003	0
WAMC057	0.96627	0.03366	0.00004	0.00004	0
WAMC063	0.19328	**0.80252**	0.00419	0.00001	0
WAMC082	0.99412	0.00583	0.00002	0.00002	0
WAMC084	0.98998	0.00999	0.00002	0.00001	0
WAMC092	0.99469	0.00527	0	0.00004	0
WAMC103	0.90599	0.09395	0.00004	0.00002	0
WAMC114	0.99055	0.00942	0.00002	0.00001	0
WAMC121	0.99527	0.0047	0.00002	0.00001	0
WAMC127	0.97302	0.02694	0.00001	0.00003	0

Sample	Coriacea-fly	Backcross-coriacea	F1	Backcross-glabra	Glabra-fly
WAMC128	*0.89917*	0.10065	0.00017	0.00001	0
WAMC141	0.97811	0.02186	0	0.00003	0
WAMC148	0.99483	0.00514	0.00003	0	0
WAMG001	0	0	0.00006	0.01718	0.98276
WAMG005	0	0	0.00012	0.05285	0.94703
WAMG008	0	0	0.00001	0.00597	0.99402
WAMG012	0	0	0	0.00164	0.99836
WAMG016	0	0	0.00002	0.00305	0.99692
WAMG020	0	0	0.00001	0.00534	0.99465
WAMG031	0	0.00001	0.00012	0.03445	0.96543
WAMG037	0	0.00001	0.00015	0.06199	0.93785
WAMG038	0	0	0.00001	0.00556	0.99443
WAMG040	0	0	0	0.00131	0.99869
WAMG043	0	0	0.00002	0.00144	0.99854
WAMG050	0	0	0	0.00401	0.99598
WAMG055	0	0	0.00001	0.00108	0.99891
WAMG062	0	0.00002	0.00035	0.10234	*0.89729*
WAMG068	0	0	0	0.00074	0.99925
WAMG075	0	0	0.00001	0.00003	0.99995
WAMG092	0	0	0.00003	0.01995	0.98003
WAMG096	0	0	0	0.00105	0.99895

APPENDIX D: Full results of genome scans

i) Among host comparisons. **ii)** Within host and among sex comparisons.
Under "Total" the first column is from DFDIST and the second column is from BAYESCAN. All other columns are results from DFDIST. Markers with no polymorphism in a particular subset are labeled as "rem" for removed. Numbers indicate significance (99%, 95%, or 90% level.) Only markers with 95% or greater probability of being outliers were included as outliers in Chapter 1.

i)

Locus	coriacea-flies	glabra-flies	Total		NC-DE	NC-NC	NC-SC	NC-EFL	SC-DE	SC-NC	SC-SC	SC-EFL	EFL-DE	EFL-NC	EFL-SC	EFL-EFL	WFL-DE	WFL-NC	WFL-SC	WFL-EFL
1	rem	90			rem	rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	Rem	
2																				
3									rem			rem	rem	rem	rem	rem	rem			rem
4													rem	rem		rem	rem			rem
5																				
6													rem		rem		rem		rem	
7		rem							rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
8	99				90				95	90	90									
9									rem			rem	rem			rem	rem			rem
10	rem				rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
11		rem											rem	rem	rem	rem	rem	rem	rem	rem
12	rem				rem			rem	rem			rem	rem			rem	rem			rem
13			99	99		99	99	95		99	99	95		90	95			90	95	
14	90	rem			rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem				
15		rem											rem	rem	rem	rem	rem		rem	rem
16	rem				rem	rem	rem	rem				rem	rem	rem	rem	rem	rem		rem	rem
17																				
18		rem											rem	rem	rem	rem	rem	rem	rem	rem

Locus	coriacea-flies	glabra-flies	Total	NC-DE	NC-NC	NC-SC	NC-EFL	SC-DE	SC-NC	SC-SC	SC-EFL	EFL-DE	EFL-NC	EFL-SC	EFL-EFL	WFL-DE	WFL-NC	WFL-SC	WFL-EFL
19				rem	rem		rem					rem	rem		rem	rem	rem		rem
20																			
21								rem	rem			rem	rem	rem	rem	rem			
22		90										rem	rem			rem	rem		
23		rem						rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
24	rem			rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem	rem
25							rem		rem		rem		rem		rem				rem
26					rem	rem	rem							rem	rem			rem	rem
27		rem						rem		rem	rem	rem	rem	rem	rem	rem		rem	rem
28	rem	95			rem		rem		rem		rem		rem		rem		rem		rem
29																			
30		rem						rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
31																			
32																			
33	rem			rem			rem	rem			rem	rem			rem				
34												rem	rem		rem	rem			
35																			
36																			
37												rem	rem			rem			
38	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem			rem
39	rem			rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem	rem
40		rem		rem	rem	rem	rem					rem	rem	rem	rem	rem	rem	rem	rem
41																			
42										90		rem		rem	rem	rem		rem	rem
43		rem										rem	rem	rem	rem	rem	rem	rem	rem
44	rem	rem		rem	rem	rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
45	90											rem	rem	rem					

Locus	coriacea-flies	glabra-flies	Total	NC-DE	NC-NC	NC-SC	NC-EFL	SC-DE	SC-NC	SC-SC	SC-EFL	EFL-DE	EFL-NC	EFL-SC	EFL-EFL	WFL-DE	WFL-NC	WFL-SC	WFL-EFL
46	rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
47		rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
48	rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
49	rem	rem		rem	rem	rem	rem	rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem
50	rem	rem		rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	rem
51	95											rem							
52					rem		rem		rem		rem								
53	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem
54		rem						rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	rem
55		rem								90		rem	rem	rem	rem	rem	rem	rem	rem
56				rem	rem		rem	rem	rem		rem	rem			rem	rem			rem
57	rem			rem	rem	rem		rem	rem	rem		rem	rem	rem		rem		rem	
58	rem				rem	rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	rem
59	rem			rem			rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	rem
60		rem		rem	rem	rem	rem					rem	rem	rem	rem	rem	rem	rem	rem
61				rem			rem	rem			rem	rem	rem	rem	rem	rem		rem	rem
62	rem	rem			rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
63		rem										rem	rem		rem	rem	rem	rem	rem
64				rem			rem					rem			rem	rem			rem
65		rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
66		90										rem	rem			rem			
67		rem						rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	rem
68		rem		rem	rem	rem	rem					rem	rem	rem	rem	rem	rem	rem	rem
69				90								rem				rem			
70	95		99 99	99	99	99	95	99	99	99	95					95	95		
71		rem											rem	rem	rem			rem	rem
72	99		99 99	99	99	99		99	99	99	95	rem					95	99	

Locus	coriacea-flies	glabra-flies	Total	NC-DE	NC-NC	NC-SC	NC-EFL	SC-DE	SC-NC	SC-SC	SC-EFL	EFL-DE	EFL-NC	EFL-SC	EFL-EFL	WFL-DE	WFL-NC	WFL-SC	WFL-EFL
73							90				90			95	95				
74		99											rem		rem				rem
75	rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem			rem
76								rem	rem		rem	rem	rem		rem	rem			rem
77											rem				rem				rem
78																			
79																			
80															rem				rem
81		rem						rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
82																rem		rem	rem
83				rem	rem							rem	rem	rem	rem	rem	rem	rem	rem
84	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem
85																			
86												rem	rem	rem	rem	rem		rem	rem
87			90	95		95				95		rem	rem	rem	rem	rem		rem	
88																rem			
89		rem						rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	rem
90	rem										rem				rem				rem
91	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem
92	99	rem										rem	rem	rem	rem	95	99	99	99
93	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem
94			99 99	99	90	99	95	99	95	99	95	95		99	95	95		99	90
97	rem									rem				rem				rem	
98	rem	rem		rem	rem	rem	rem	rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem
99																rem			
100								rem				rem	rem		rem	rem			rem
101	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem

Locus	coriacea-flies	glabra-flies	Total	NC-DE	NC-NC	NC-SC	NC-EFL	SC-DE	SC-NC	SC-SC	SC-EFL	EFL-DE	EFL-NC	EFL-SC	EFL-EFL	WFL-DE	WFL-NC	WFL-SC	WFL-EFL
102																			
103																			
104	rem						rem				rem				rem				rem
105		rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	rem
106																			
107																			
108		rem			rem	rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
109		95																	
110																			
111	99			90											rem			90	
112												rem	rem		rem				
113															rem				
114	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem
115			99 99	99	99	99	95	99	99	99	99		95				95	95	
116	99	rem			rem	rem	rem	rem	rem	rem	rem			95		rem	rem	rem	rem
117	rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
118			99 99	99	99	99	99	99	99	99	99		90	99	90	95	99	99	99
119		rem						rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
120	rem	rem		rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
121				rem				rem				rem				rem			
122	95			rem			rem												
123	rem	rem		rem	rem	rem	rem	rem		rem	rem	rem	rem	rem	rem	rem		rem	rem
124												rem				rem			rem
125																			
126												rem	rem			rem			
127	rem	rem		rem	rem	rem	rem	rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem
128	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem

Locus	coriacea-flies	glabra-flies	Total	NC-DE	NC-NC	NC-SC	NC-EFL	SC-DE	SC-NC	SC-SC	SC-EFL	EFL-DE	EFL-NC	EFL-SC	EFL-EFL	WFL-DE	WFL-NC	WFL-SC	WFL-EFL
129		rem						rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
130	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem
131															rem				rem
132																			
134		rem		rem	rem	rem						rem	rem	rem	rem	rem	rem	rem	rem
135				rem	rem			rem	rem			rem	rem		rem	rem	rem		rem
136		rem										rem	rem	rem	rem	rem	rem	rem	rem
137																			
138																			
139			90						90										
140												rem							
141	rem	rem		rem	rem	rem	rem	rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem
142	rem					rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	rem
143		rem						rem			rem	rem			rem	rem			rem
144	90	rem	90	90				90	95	99	95		90	95			99	99	
145		rem		rem	rem	rem	rem					rem	rem	rem	rem	rem	rem	rem	rem
146		rem		rem	rem	rem	rem					rem	rem	rem	rem	rem	rem	rem	rem
147	rem		90			95	90												
148	95	rem		rem	rem	rem	rem					rem	rem	rem	rem		90	95	
149																			
150																			
151																			
152																			
153												rem				rem			rem
154												rem			rem	rem			rem
155		rem										rem	rem	rem	rem	rem		rem	rem
156												rem							

Locus	coriacea-flies	glabra-flies	Total	NC-DE	NC-NC	NC-SC	NC-EFL	SC-DE	SC-NC	SC-SC	SC-EFL	EFL-DE	EFL-NC	EFL-SC	EFL-EFL	WFL-DE	WFL-NC	WFL-SC	WFL-EFL
157	rem			rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	rem
158	rem	rem		rem	rem	rem	rem	rem	rem	rem		rem	rem	rem	rem	rem	rem	rem	rem
159							rem				rem		rem		rem				rem
160		rem										rem	rem	rem	rem	rem	rem	rem	rem
161		rem						rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
162							rem						rem		rem				rem
163	rem			rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem	rem
164																			
165															rem				
166	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem			rem
167	95	99					90				90			95					
168	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem			rem
169															rem				rem
170	90																		
171												rem			rem				
172												rem			rem				
173	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem			rem
174								rem			rem	rem			rem				
175															rem				
176		rem						rem		rem	rem	rem	rem	rem	rem	rem		rem	rem
177																			
178												rem			rem	rem			rem
179	rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
180													90	95					
181		90										rem							
182	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem
183	rem			rem		rem		rem	rem	rem		rem	rem	rem		rem		rem	

Locus	coriacea-flies	glabra-flies	Total	NC-DE	NC-NC	NC-SC	NC-EFL	SC-DE	SC-NC	SC-SC	SC-EFL	EFL-DE	EFL-NC	EFL-SC	EFL-EFL	WFL-DE	WFL-NC	WFL-SC	WFL-EFL
184		99													rem				rem
185				rem		rem						rem	rem	rem	rem	rem		rem	rem
186		rem		rem	rem	rem	rem					rem	rem	rem	rem	rem	rem	rem	rem
187		rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	rem
188	rem							rem	rem		rem	rem	rem		rem	rem			rem
189				rem			rem	rem			rem	rem	rem	rem	rem	rem		rem	rem
190					rem	rem						rem	rem	rem	rem	rem	rem	rem	rem
191												rem	rem		rem	rem			rem
192												rem			rem				
193		95														rem			
194														rem	rem			rem	rem
195		99																	rem
196	90																		
197		rem		rem	rem	rem	rem					rem	rem	rem	rem	rem	rem	rem	rem
199											rem				rem				rem
200		rem	95 95		90	95						rem	rem	rem	rem		90	95	
201												rem			rem				
202																			
203	rem	rem		rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
204	rem		99 95	95	95	90	rem	95	90		rem				rem				rem
205						rem	rem							rem	rem			rem	rem
206																			
207																rem			rem
208				rem			rem	rem			rem	rem		rem	rem	rem			rem
209																			
210												rem			rem				rem
211																			

Locus	coriacea-flies	glabra-flies	Total		NC-DE	NC-NC	NC-SC	NC-EFL	SC-DE	SC-NC	SC-SC	SC-EFL	EFL-DE	EFL-NC	EFL-SC	EFL-EFL	WFL-DE	WFL-NC	WFL-SC	WFL-EFL
212		rem							rem		rem	rem	rem		rem	rem	rem		rem	rem
213			95	95	95	95	90	95					95	95	95	95	90	95	99	95
214	rem							rem								rem				rem
215		rem											rem	rem	rem	rem	rem		rem	rem
216		rem							rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem
217												90								
218													rem			rem	rem			rem
219																				
220																rem				
221		rem			rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
222	rem				rem			rem	rem			rem	rem			rem	rem			rem
223		rem											rem	rem	rem	rem				
224																				
225	99	rem			rem	rem	rem	rem	rem	rem	rem	rem			95		rem		rem	rem
226	99	99												95	95	95				
227	rem		99	99		95	99	90		90	99									
228																rem	rem			rem
229		99											90			rem	90			rem
230																rem				rem
231			95	95		90	95				95						rem		rem	rem
232																				
233													rem							
234																				
235	rem				rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem
236													95	90	95	95			90	90
237					rem				rem				rem				rem			
238			95							90	95		rem					90	90	

Locus	coriacea-flies	glabra-flies	Total	NC-DE	NC-NC	NC-SC	NC-EFL	SC-DE	SC-NC	SC-SC	SC-EFL	EFL-DE	EFL-NC	EFL-SC	EFL-EFL	WFL-DE	WFL-NC	WFL-SC	WFL-EFL
239			90	95	95														
240																	95		
241	rem	99			rem	rem	rem		rem	rem	rem		rem	rem	rem			rem	rem
242			99 95	95	99	90		95	99	90									
243	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem	rem		rem
244	rem			rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	rem
245	95												95	95	90	rem	rem		rem
246			99 99	95	99	95	95	95	99	99	95		95				95	95	
247		rem						rem	rem		rem	rem		rem	rem	rem	rem	rem	rem
248												rem	rem		rem				
249																			
250																rem			rem
251																			
252	rem			rem	rem		rem	rem	rem		rem	rem	rem		rem	rem			rem
253																			rem
254	rem			rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	rem
255	rem	90	99 99	90	99	99	99		99	99	99		90	99	95		99	99	99
256	rem			rem				rem				rem				rem			
257	rem			rem			rem	rem	rem		rem	rem	rem		rem	rem			rem
258		rem						rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
259		95						95		90		90		90	rem	90	90	95	rem
260															rem				
261																			
262				rem	rem		rem		rem		rem		rem		rem	rem	rem		rem
263		rem						rem	rem		rem	rem	rem	rem	rem	rem	rem	rem	rem
264																			
265																			

Locus	coriacea-flies	glabra-flies	Total	NC-DE	NC-NC	NC-SC	NC-EFL	SC-DE	SC-NC	SC-SC	SC-EFL	EFL-DE	EFL-NC	EFL-SC	EFL-EFL	WFL-DE	WFL-NC	WFL-SC	WFL-EFL
266															rem				rem
267												rem	rem		rem	rem	rem		rem
268																			
269		rem		rem		rem	rem					rem	rem	rem	rem	rem		rem	rem

ii)

	Within coriacea-flies					Within glabra-flies				Sex			
Locus	**NC-EFL**	**NC-WFL**	**SC-EFL**	**SC-WFL**	**EFL-WFL**	**DE-SC**	**DE-EFL**	**NC-EFL**	**SC-EFL**	**coriacea-flies**	**glabra-flies**	**Total**	
1	rem	rem	rem	rem	rem	rem		90	99	rem			
2										99	99	99	99
3			rem		rem	rem	rem		rem				
4					rem								
5													
6	rem	rem			rem								
7			rem	rem	rem	rem	rem	rem	rem		rem		
8	99	rem	99	rem	95								
9			rem	rem	rem								
10	rem	rem	rem	rem	rem	rem	rem			rem			
11					rem	rem	rem	rem	rem		rem		
12	rem	rem	rem	rem	rem		rem			rem			
13													
14	rem	95	rem	95		rem	rem		rem		rem		
15					rem	rem	rem		rem		rem		
16	rem	rem	rem	rem	rem		rem		rem	rem			
17													
18					rem	rem	rem	rem	rem		rem		

	Within coriacea-flies					Within glabra-flies				Sex			
Locus	**NC-EFL**	**NC-WFL**	**SC-EFL**	**SC-WFL**	**EFL-WFL**	**DE-SC**	**DE-EFL**	**NC-EFL**	**SC-EFL**	**coriacea-flies**	**glabra-flies**	**Total**	
19	rem	rem			rem		rem	rem					
20										99	99	99	99
21			rem		rem	rem	rem						
22					rem			90			95	95	
23			rem	rem	rem	rem	rem	rem	rem		rem		
24	rem	rem	rem	rem	rem	rem	rem		rem	rem			
25	rem		rem	rem	rem								
26	rem	rem			rem				rem				
27			rem	rem	rem	rem	rem		rem		rem		
28	rem	rem	rem	rem	rem	90		rem					
29						90							
30			rem	rem	rem	rem	rem	rem	rem		rem		
31				rem	rem								
32										99	99	99	99
33	rem	rem	rem	rem	rem					rem			
34					rem		rem						
35					rem								
36					rem								
37					rem								
38	rem	rem	rem	rem	rem		rem			rem			
39	rem	rem	rem	rem	rem	rem	rem		rem	rem			
40	rem	rem			rem	rem	rem	rem	rem		rem		
41										99	99	99	99
42					rem	rem	rem		rem				
43					rem	rem	rem	rem	rem	95	rem	95	
44	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		

	Within coriacea-flies					Within glabra-flies				Sex		
Locus	**NC-EFL**	**NC-WFL**	**SC-EFL**	**SC-WFL**	**EFL-WFL**	**DE-SC**	**DE-EFL**	**NC-EFL**	**SC-EFL**	**coriacea-flies**	**glabra-flies**	**Total**
45	rem	90	rem	90								
46	rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	
47	rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	
48	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	
49	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
50	rem	rem	rem	rem	rem	rem	rem		rem	rem	rem	
51			rem	95								
52	rem	rem	rem	rem							90	
53	rem	rem	rem	rem	rem		rem	rem		rem		
54			rem	rem	rem	rem	rem	rem	rem		rem	
55					rem	rem	rem	rem	rem		rem	
56	rem	rem	rem	rem			rem					
57	rem	rem	rem	rem	rem	rem			90	rem		
58	rem	rem	rem	rem	rem			rem	rem	rem		
59	rem	rem	rem	rem	rem	rem	rem		rem	rem		
60	rem	rem			rem	rem	rem	rem	rem		rem	
61	rem	rem	rem	rem	rem	rem	rem		rem			
62	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
63					rem	rem	rem	rem	rem	90	rem	
64	rem	rem			rem		rem					
65	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem
66					rem			90				
67			rem	rem	rem	rem	rem		rem		rem	
68		rem			rem	rem	rem	rem	rem		rem	
69		90									95	
70	95	rem	90	rem		90					99	

	Within coriacea-flies					Within glabra-flies				Sex		
Locus	**NC-EFL**	**NC-WFL**	**SC-EFL**	**SC-WFL**	**EFL-WFL**	**DE-SC**	**DE-EFL**	**NC-EFL**	**SC-EFL**	**coriacea-flies**	**glabra-flies**	**Total**
71						rem	rem	rem	rem			
72	99		99		95				90			
73												
74					rem	95						
75	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	
76			rem	rem	rem							
77			rem		rem							
78					rem							
79					rem							
80					rem							
81			rem	rem	rem	rem	rem	rem	rem		rem	
82						rem	rem		rem			
83	rem	rem			rem	rem	rem					
84	rem	rem	rem	rem	rem		rem	rem		rem		
85			rem	rem	rem							
86	rem	rem	rem	rem	rem							
87	90				rem	rem	rem		rem			
88					rem					90		
89			rem	rem	rem	rem	rem	rem	rem		rem	
90	rem	rem	rem	rem	rem					rem		
91	rem	rem	rem	rem	rem		rem	rem		rem		
92		99		99	99	rem	rem	rem	rem		rem	
93	rem	rem	rem	rem	rem		rem	rem		rem		
94					rem		rem					
97	rem	rem	rem	rem	rem					rem		
98	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem

	Within coriacea-flies						Within glabra-flies			Sex			
Locus	**NC-EFL**	**NC-WFL**	**SC-EFL**	**SC-WFL**	**EFL-WFL**	**DE-SC**	**DE-EFL**	**NC-EFL**	**SC-EFL**	**coriacea-flies**	**glabra-flies**	**Total**	
99				rem	rem					99	95	99	90
100			rem	rem	rem		rem						
101	rem	rem	rem	rem	rem		rem	rem		rem			
102					rem								
103													
104	rem	rem	rem	rem	rem					rem			
105	rem		rem	rem	rem	rem	rem	rem	rem		rem		
106													
107					rem								
108	rem	rem	rem	rem	rem	rem	rem	rem	rem		rem		
109							90	99	95	95			
110													
111	rem	99		95	90	90							
112							rem						
113					rem					99		95	
114	rem	rem	rem	rem	rem		rem	rem		rem			
115							rem	rem		90			
116	99	rem	95	rem		rem	rem	rem	rem		rem		
117	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem			
118		rem	rem	rem	rem	rem	rem		rem		rem		
119			rem	rem	rem	rem	rem	rem	rem		rem		
120	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		
121	rem	rem	rem	rem									
122	95	95					rem						
123	rem	rem	rem	rem	rem	rem	rem		rem	rem	rem		
124					rem		rem			95	99	99	

	Within coriacea-flies					Within glabra-flies				Sex			
Locus	**NC-EFL**	**NC-WFL**	**SC-EFL**	**SC-WFL**	**EFL-WFL**	**DE-SC**	**DE-EFL**	**NC-EFL**	**SC-EFL**	**coriacea-flies**	**glabra-flies**	**Total**	
125										99	99	99	99
126													
127	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		
128	rem	rem	rem	rem	rem		rem	rem		rem			
129			rem	rem	rem	rem	rem	rem	rem		rem		
130	rem	rem	rem	rem	rem		rem	rem		rem			
131	rem	rem	rem	rem	rem								
132										99	99	99	99
134	rem	rem			rem	rem	rem		rem		rem		
135	rem	rem	rem	rem	rem		rem						
136					rem	rem	rem	rem	rem		rem		
137				90						99	99	99	99
138													
139			rem	rem	rem								
140					rem								
141	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		
142	rem	rem	rem	rem	rem	rem	rem		rem	rem			
143			rem	rem	rem		rem						
144		90			rem								
145	rem	rem			rem	rem	rem	rem	rem		rem		
146	rem	rem			rem	rem	rem	rem	rem		rem		
147	rem	rem	rem	rem	rem					rem			
148	rem	95		90		rem	rem	rem	rem		rem		
149					rem				90		90		
150													
151													

	Within coriacea-flies					Within glabra-flies				Sex		
Locus	**NC-EFL**	**NC-WFL**	**SC-EFL**	**SC-WFL**	**EFL-WFL**	**DE-SC**	**DE-EFL**	**NC-EFL**	**SC-EFL**	**coriacea-flies**	**glabra-flies**	**Total**
152												
153					rem		rem					
154					rem		rem					
155					rem	rem	rem		rem		rem	
156												
157	rem	rem	rem	rem	rem	rem	rem			rem		
158	rem	rem	rem	rem	rem	rem	rem		rem	rem	rem	rem
159	rem		rem		rem							
160					rem	rem	rem	rem	rem	95	rem	
161			rem	rem	rem	rem	rem	rem	rem		rem	
162	rem	rem			rem							
163	rem	rem	rem	rem	rem	rem	rem		rem	rem		
164											90	
165					rem						90	
166	rem	rem	rem	rem	rem		rem			rem		
167	95		99				90		99			
168	rem	rem	rem	rem	rem					rem		
169					rem							
170		95						95				
171					rem							
172					rem							
173	rem	rem	rem	rem	rem		rem	rem		rem		
174				90			rem					
175	90				rem							
176			rem	rem	rem	rem	rem		rem		rem	
177												

	Within coriacea-flies					Within glabra-flies				Sex			
Locus	**NC-EFL**	**NC-WFL**	**SC-EFL**	**SC-WFL**	**EFL-WFL**	**DE-SC**	**DE-EFL**	**NC-EFL**	**SC-EFL**	**coriacea-flies**	**glabra-flies**	**Total**	
178					rem		rem						
179	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		
180										99			
181										90			
182	rem	rem	rem	rem	rem		rem	rem		rem			
183	rem	rem	rem	rem	rem	rem			90	rem			
184					rem	99	90						
185	rem	rem			rem	rem	rem		rem				
186	rem	rem			rem	rem	rem	rem	rem	rem	rem	rem	
187	rem		rem		rem	rem	rem	rem	rem		rem		
188	rem	rem	rem	rem	rem					rem	95	95	
189	rem	rem	rem	rem	rem	rem	rem		rem				
190	rem	rem			rem	rem			rem				
191					rem		rem			99		95	
192					rem					99	99	99	99
193					rem			95	99	99	99	99	95
194					rem				rem				
195						90							
196		90		95									
197	rem	rem			rem	rem	rem	rem	rem	rem	rem	rem	
199				rem	rem					99		95	
200						rem	rem	rem	rem		rem		
201						rem	rem		rem				
202													
203	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem	rem		
204	rem	rem	rem	rem	rem					rem			

	Within coriacea-flies					Within glabra-flies				Sex		
Locus	**NC-EFL**	**NC-WFL**	**SC-EFL**	**SC-WFL**	**EFL-WFL**	**DE-SC**	**DE-EFL**	**NC-EFL**	**SC-EFL**	**coriacea-flies**	**glabra-flies**	**Total**
205	rem	rem			rem				rem			
206					rem							
207		90					rem			90		
208	rem		rem		rem	rem	rem		rem			
209												
210					rem		rem					
211												
212				rem	rem	rem	rem		rem		rem	
213					rem							
214	rem	rem	rem	rem	rem					rem		
215					rem	rem	rem	rem	rem		rem	
216			rem	rem	rem	rem	rem	rem	rem		rem	
217												
218			rem	rem	rem							
219												
220					rem							
221	rem	rem	rem	rem	rem	rem	rem	rem	rem		rem	
222	rem	rem	rem	rem	rem		rem			rem		
223						rem	rem	rem	rem		rem	
224												
225	99	rem	95			rem	rem	rem	rem		rem	
226		90	95		95	90	90					
227	rem	rem	rem	rem	rem					rem		
228					rem		rem					
229		90			rem	99	99	90				
230					rem						95	

	Within coriacea-flies					Within glabra-flies				Sex			
Locus	**NC-EFL**	**NC-WFL**	**SC-EFL**	**SC-WFL**	**EFL-WFL**	**DE-SC**	**DE-EFL**	**NC-EFL**	**SC-EFL**	**coriacea-flies**	**glabra-flies**	**Total**	
231		90		95		rem	rem		rem		rem		
232						95							
233					90								
234		95											
235	rem	rem	rem	rem	rem		rem	rem		rem			
236	90		90		rem		rem						
237	rem	rem	rem	rem	rem								
238	90		90		90					99		90	
239					rem								
240					rem								
241	rem	rem	rem	rem	rem	95			rem	rem			
242		rem	rem	rem	rem			90					
243	rem	rem	rem	rem	rem		rem	rem		rem			
244	rem	rem	rem	rem	rem	rem	rem		rem	rem			
245	99		90		95		rem	rem					
246							rem	rem					
247			rem	rem	rem	rem	rem	rem	rem		rem		
248							rem						
249									90	99	99	99	99
250							rem			99		99	99
251									95	99	99	99	99
252	rem	rem	rem	rem	rem		rem			rem			
253										90			
254	rem	rem	rem	rem	rem	rem	rem		rem	rem			
255	rem	rem	rem	rem	rem					rem			
256	rem	rem	rem	rem	rem					rem			

	Within coriacea-flies						Within glabra-flies			Sex			
Locus	**NC-EFL**	**NC-WFL**	**SC-EFL**	**SC-WFL**	**EFL-WFL**	**DE-SC**	**DE-EFL**	**NC-EFL**	**SC-EFL**	**coriacea-flies**	**glabra-flies**	**Total**	
257	rem	rem	rem	rem	rem		rem			rem			
258			rem	rem	rem	rem	rem		rem		rem		
259					rem		95		99				
260					rem					95	99	99	90
261										99	99	99	99
262	rem	rem		rem	rem		rem	rem		rem			
263			rem	rem	rem	rem	rem	rem	rem		rem		
264													
265													
266					rem	90							
267					rem		rem	rem					
268													
269	rem	rem			rem	rem	rem		rem				

APPENDIX E: Classification of plant samples based on the results of NEWHYBRIDS and STRUCTURE analyses

Highest probabilities are indicated in bold. Using a cutoff of 0.90 for belonging to a parental group, both analyses result in the same the final classification, given in the last column.

	NEWHYBRIDS					STRUCTURE		
Sample	*I. coriacea*	backcross *coriacea*	F1	backcross *glabra*	*I. glabra*	*I. coriacea*	*I. glabra*	Classification
P152C012	**1**	0	0	0	0	**0.9999**	0.0001	*I. coriacea*
P152C013	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
P152C014	**1**	0	0	0	0	**0.996**	0.004	*I. coriacea*
P152C031	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
P152C042	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
P152C077	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
P152C092	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
P152C099	**1**	0	0	0	0	**0.9987**	0.0013	*I. coriacea*
P152C122	**1**	0	0	0	0	**0.998**	0.002	*I. coriacea*
P152C127	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
P152C130	**1**	0	0	0	0	**1**	0	*I. coriacea*
P152C133	**1**	0	0	0	0	**0.9991**	0.0009	*I. coriacea*
P152C222	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
P152C234	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
P152C240	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
P152C247	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
P152C254	**1**	0	0	0	0	**0.994**	0.006	*I. coriacea*
P152C271	**0.99996**	0.00004	0	0	0	**0.9877**	0.0123	*I. coriacea*
P152C272	**1**	0	0	0	0	**1**	0	*I. coriacea*
P152C280	**1**	0	0	0	0	**0.997**	0.003	*I. coriacea*
P152C288	**0.84829**	0.1517	0.00001	0	0	**0.8776**	0.1224	late Bx *I.coriacea*
P152CE02	**0.7758**	0.22419	0	0	0	**0.833**	0.167	late Bx *I.coriacea*
P152CE06	**1**	0	0	0	0	**0.998**	0.002	*I. coriacea*
P152G027	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
P152G167	0	0	0	0	**1**	0.0062	**0.9938**	*I. glabra*
P152G168	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
P152G172	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
P152G174	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
P152G180	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
P152G183	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
P152G199	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
P152GE01	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
P152GE02	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PBOBC006	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PBOBC016	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PBOBC046	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PBOBC047	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PBOBC048	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PBOBC061	**1**	0	0	0	0	**0.998**	0.002	*I. coriacea*
PBOBC084	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PBOBC092	**1**	0	0	0	0	**1**	0	*I. coriacea*
PBOBC142	**1**	0	0	0	0	**0.9992**	0.0008	*I. coriacea*

Sample	NewHybrids					Structure		Classification
	I. coriacea	backcross *coriacea*	F1	backcross *glabra*	*I. glabra*	*I. coriacea*	*I. glabra*	
PBOBC149	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PBOBC181	**0.99999**	0.00001	0	0	0	**0.9632**	0.0368	*I. coriacea*
PBOBC187	**1**	0	0	0	0	**0.997**	0.003	*I. coriacea*
PBOBC191	**0.75963**	0.24037	0	0	0	**0.8573**	0.1427	late Bx *I.coriacea*
PBOBC198	**1**	0	0	0	0	**0.9992**	0.0008	*I. coriacea*
PBOBC228	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PBOBC240	**1**	0	0	0	0	**0.9998**	0.0002	*I. coriacea*
PBOBCE04	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PBOBCE05	0	0.00662	**0.99339**	0	0	**0.552**	0.448	F1 hybrid
PBOBG011	0	0	0	0.00009	**0.99991**	0.0584	**0.9416**	*I. glabra*
PBOBG028	0	0	0	0	**1**	0.004	**0.996**	*I. glabra*
PBOBG067	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PBOBG159	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PBOBG169	0	0	0	0	**1**	0.007	**0.993**	*I. glabra*
PBOBG170	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PBOBG174	0	0	0	0	**1**	0.006	**0.994**	*I. glabra*
PBOBG182	0	0	0	0	**1**	0.005	**0.995**	*I. glabra*
PBOBG190	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PBOBG198	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PBOBG205	0	0	0	0	**1**	0.003	**0.997**	*I. glabra*
PBOBGE01	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PBOBGE17	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCATC093	**0.99848**	0.00152	0	0	0	**0.9813**	0.0187	*I. coriacea*
PCATC115	0.00001	**0.99938**	0.00061	0	0	**0.7431**	0.2569	Bx *I. coriacea*
PCATC185	**1**	0	0	0	0	**0.998**	0.002	*I. coriacea*
PCATC197	**1**	0	0	0	0	**0.997**	0.003	*I. coriacea*
PCATC204	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PCATC212	**1**	0	0	0	0	**0.989**	0.011	*I. coriacea*
PCATC219	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PCATC225	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PCATC240	**0.99999**	0.00001	0	0	0	**0.98**	0.02	*I. coriacea*
PCATC245	**1**	0	0	0	0	**0.997**	0.003	*I. coriacea*
PCATG107	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCATG114	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCATG134	0	0	0	0	**1**	0.005	**0.995**	*I. glabra*
PCATG137	0	0	0	0	**1**	0.002	**0.998**	*I. glabra*
PCATG143	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCATG148	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCATG151	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCATGE09	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCATGE17	0	0	0	0	**1**	0.002	**0.998**	*I. glabra*
PCHEG003	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCHEG033	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCHEG047	0	0	0	0	**1**	0.003	**0.997**	*I. glabra*
PCHEG058	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCHEG089	0	0	0	0	**1**	0.0226	**0.9774**	*I. glabra*
PCHEG093	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCHEG114	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCHEG119	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCRGG001	0	0	0	0	**1**	0.0206	**0.9794**	*I. glabra*
PCRGG007	0	0	0	0	**1**	0.002	**0.998**	*I. glabra*
PCRGG008	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*

Sample	NewHybrids					Structure		Classification
	I. coriacea	backcross *coriacea*	F1	backcross *glabra*	*I. glabra*	*I. coriacea*	*I. glabra*	
PCRGG010	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCRGG013	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCRGG014	0	0	0	0	**1**	0.0186	**0.9814**	*I. glabra*
PCRGG017	0	0	0	0	**1**	0.008	**0.992**	*I. glabra*
PCRGGE24	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCRGGE32	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PCRGGE36	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PEAVG001	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PEAVG002	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PEAVG004	0	0	0	0.00012	**0.99988**	0.0514	**0.9486**	*I. glabra*
PEAVGE02	0	0	0	0	**1**	0.004	**0.996**	*I. glabra*
PEAVGE03	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PEAVGE06	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PEAVH003	0	0	0	0.00004	**0.99996**	0.062	**0.938**	*I. glabra*
PEAVH004	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PEAVHE01	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PEAVHE02	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PGDSC003	**1**	0	0	0	0	**0.9988**	0.0012	*I. coriacea*
PGDSC009	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PGDSC012	**1**	0	0	0	0	**0.998**	0.002	*I. coriacea*
PGDSC024	**1**	0	0	0	0	**0.996**	0.004	*I. coriacea*
PGDSC036	**1**	0	0	0	0	**0.997**	0.003	*I. coriacea*
PGDSC055	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PGDSC057	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PGDSCE01	**1**	0	0	0	0	**0.997**	0.003	*I. coriacea*
PGDSCE02	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PGDSG018	0	0	0	0.00005	**0.99995**	0.0565	**0.9435**	*I. glabra*
PGDSG020	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PGDSG021	0	0	0	0	**1**	0.0272	**0.9728**	*I. glabra*
PGDSG026	0	0	0	0	**1**	0.002	**0.998**	*I. glabra*
PGDSG032	0	0	0	0	**1**	0.006	**0.994**	*I. glabra*
PGDSG037	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PGDSG046	0	0	0	0	**1**	0.007	**0.993**	*I. glabra*
PGDSGE05	0	0	0	0.00002	**0.99998**	0.0583	**0.9417**	*I. glabra*
PGDSGE12	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PHUNC001	**0.99999**	0.00001	0	0	0	**0.994**	0.006	*I. coriacea*
PHUNC003	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PHUNC006	**0.99996**	0.00004	0	0	0	**0.9839**	0.0161	*I. coriacea*
PHUNC010	**1**	0	0	0	0	**0.998**	0.002	*I. coriacea*
PHUNC012	0.00762	**0.97475**	0.01763	0	0	**0.7429**	0.2571	Bx *I. coriacea*
PHUNC014	**1**	0	0	0	0	**0.998**	0.002	*I. coriacea*
PHUNCE04	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PHUNCE06	**1**	0	0	0	0	**0.996**	0.004	*I. coriacea*
PHUNCE08	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PHUNG002	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PHUNGE01	0	0	0	0	**1**	0.009	**0.991**	*I. glabra*
PHUNGE05	0	0.00008	**0.99989**	0.00002	0	0.4954	0.5046	F1 hybrid
PHUNGE07	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PHUNGE09	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PHUNGE11	0	0	0	0	**1**	0.0019	**0.9981**	*I. glabra*
PHUNGE14	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PHUNGE15	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*

Sample	NewHybrids					Structure		Classification
	I. coriacea	backcross *coriacea*	F1	backcross *glabra*	*I. glabra*	*I. coriacea*	*I. glabra*	
PSISC001	**1**	0	0	0	0	**0.998**	0.002	*I. coriacea*
PSISC009	**0.96664**	0.03336	0	0	0	**0.9164**	0.0836	*I. coriacea*
PSISC010	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PSISC013	**1**	0	0	0	0	**0.998**	0.002	*I. coriacea*
PSISC025	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PSISC026	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PSISC028	**0.99958**	0.00042	0	0	0	**0.9851**	0.0149	*I. coriacea*
PSISC033	**1**	0	0	0	0	**0.997**	0.003	*I. coriacea*
PSISCE36	**0.99408**	0.00592	0	0	0	**0.9033**	0.0967	*I. coriacea*
PSISCE37	**0.99999**	0.00001	0	0	0	**0.992**	0.008	*I. coriacea*
PSISG006	0	0	0	0	**1**	0.0016	**0.9984**	*I. glabra*
PSISG010	0	0	0	0	**1**	0.002	**0.998**	*I. glabra*
PSISG032	0	0	0	0.00004	**0.99996**	0.0484	**0.9516**	*I. glabra*
PSISG048	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PSISG057	0	0	0	0	**1**	0.005	**0.995**	*I. glabra*
PSISG063	0	0	0	0	**1**	0.002	**0.998**	*I. glabra*
PSISG076	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PSISGE16	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PSOPC001	**1**	0	0	0	0	**0.997**	0.003	*I. coriacea*
PSOPC005	0	0.31468	**0.68532**	0	0	**0.656**	0.344	F1 / Bx *I. coriacea*
PSOPCE01	**1**	0	0	0	0	**0.998**	0.002	*I. coriacea*
PSOPCE02	**0.99879**	0.00121	0	0	0	**0.9689**	0.0311	*I. coriacea*
PSOPCE03	**1**	0	0	0	0	**0.9929**	0.0071	*I. coriacea*
PSOPGE02	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PWAMC013	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PWAMC014	**1**	0	0	0	0	**0.997**	0.003	*I. coriacea*
PWAMC034	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PWAMC036	**1**	0	0	0	0	**0.998**	0.002	*I. coriacea*
PWAMC040	**0.99999**	0.00001	0	0	0	**0.994**	0.006	*I. coriacea*
PWAMC046	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PWAMC057	**1**	0	0	0	0	**0.9956**	0.0044	*I. coriacea*
PWAMC063	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PWAMC084	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PWAMC090	0.01321	**0.98544**	0.00135	0	0	**0.7617**	0.2383	Bx *I. coriacea*
PWAMC106	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PWAMC113	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PWAMC121	**0.99999**	0.00001	0	0	0	**0.983**	0.017	*I. coriacea*
PWAMC123	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PWAMC128	**1**	0	0	0	0	**0.991**	0.009	*I. coriacea*
PWAMC141	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PWAMC144	**1**	0	0	0	0	**1**	0	*I. coriacea*
PWAMC148	**1**	0	0	0	0	**1**	0	*I. coriacea*
PWAMCE04	**1**	0	0	0	0	**0.999**	0.001	*I. coriacea*
PWAMCE07	**1**	0	0	0	0	**0.9967**	0.0033	*I. coriacea*
PWAMG011	0	0	0.00029	0.10433	**0.89538**	0.1699	**0.8301**	late Bx *I. glabra*
PWAMG079	0	0	0	0	**1**	0.0181	**0.9819**	*I. glabra*
PWAMG091	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PWAMG093	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PWAMG094	0	0	0	0	**1**	0.002	**0.998**	*I. glabra*
PWAMG096	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PWAMG097	0	0	0	0	**1**	0.002	**0.998**	*I. glabra*
PWAMG098	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*

Sample	NewHybrids					Structure		Classification
	I. coriacea	backcross *coriacea*	F1	backcross *glabra*	*I. glabra*	*I. coriacea*	*I. glabra*	
PWAMGE02	0	0	0	0	**1**	0.009	**0.991**	*I. glabra*
PWAMGE08	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*
PWAMGE15	0	0	0	0	**1**	0.0418	**0.9582**	*I. glabra*
PWAMGE19	0	0	0	0	**1**	0.001	**0.999**	*I. glabra*

APPENDIX F: Estimated hybrid indices of flies

Hybrid index calculated using package INTROGRESS using method described in Buerkle (2005). Flies with a 0.99 or higher membership in a parental type using NEWHYBRIDS used as training samples. Values of 0 correspond to coriacea-flies and 1 to glabra-flies.

Sample	Lower limit 95% CI	Hybrid Index	Upper limit 95% CI
152C004	0.04024	0.177669	0.367981
152C013	0	0	0.164214
152C026	0.293484	0.485933	0.686612
152C031	0.225633	0.388817	0.569845
152C032	0.041704	0.172718	0.366856
152C039	0	0.067773	0.273666
152C042	0.110608	0.274485	0.472594
152C059	0	0	0.067859
152C061	0.13374	0.268919	0.434151
152C062	0	0	0.097234
152C077	0.225287	0.400157	0.58788
152C092	0.027973	0.105241	0.242405
152C096	0.002934	0.048775	0.177236
152C102	0.264345	0.449763	0.647324
152C123	0.016885	0.095757	0.251175
152C127	0.149408	0.301338	0.482423
152C130	0.072599	0.213317	0.394513
152C142	0.175531	0.347175	0.539385
152C143	0	0	0.116087
152C190	0	0	0.110181
152C223	0.119151	0.290581	0.489884
152C248	0	0	0.068257
152C258	0.04123	0.176185	0.37407
152C264	0.04108	0.141983	0.302539
152C271	0.00786	0.096404	0.276939
152C273	0.017407	0.151249	0.338493
152C288	0.28823	0.481623	0.683161
BOBC006	0.059464	0.203115	0.396588
BOBC007	0	0	0.119467
BOBC012	0	0	0.074828
BOBC019	0	0	0.076621
BOBC023	0	0	0.112447
BOBC037	0	0	0.102516
BOBC039	0	0	0.092464
BOBC046	0	0	0.207053
BOBC049	0	0	0.055993
BOBC076	0.084266	0.243605	0.440171

Sample	Lower limit 95% CI	Hybrid Index	Upper limit 95% CI
BOBC084	0	0	0.076003
BOBC127	0	0	0.21199
BOBC128	0.014799	0.087033	0.238572
BOBC130	0	0	0.096036
BOBC149	0.019763	0.147619	0.342668
BOBC196	0.120675	0.317181	0.533168
BOBC198	0	0.103105	0.316278
BOBC230	0.023717	0.141591	0.321405
BOBC243	0.048937	0.165948	0.340814
CATC004	0	0	0.107374
CATC010	0.086385	0.254633	0.45814
CATC049	0	0.009359	0.16693
CATC051	0.006038	0.084118	0.256804
CATC105	0	0	0.106245
CATC115	0.099277	0.276397	0.483591
CATC119	0.237448	0.432325	0.636585
CATC124	0.049085	0.209599	0.422755
CATC135	0.105537	0.294366	0.512135
CATC145	0.153612	0.322014	0.518148
CATC158	0.108502	0.294497	0.505593
CATC159	0.04206	0.184082	0.383859
CATC168	0.153744	0.326972	0.527042
CATC172	0.360713	0.541901	0.723648
CATC176	0.027425	0.117041	0.268919
CATC179	0.182266	0.369523	0.573128
CATC183	0	0	0.08571
CATC189	0	0.067461	0.29542
GDSC056	0.291795	0.488745	0.690856
GDSC065	0.092554	0.224007	0.404764
HUNC002	0	0	0.171713
HUNC003	0.036956	0.137402	0.304285
HUNC006	0.226444	0.399493	0.588301
HUNC007	0.07045	0.195317	0.371834
HUNC009	0.280749	0.470731	0.666317
HUNC014	0.282814	0.481214	0.682191
SISC004	0.107627	0.257936	0.455074
SISC014	0.178078	0.355346	0.557794
SISC030	0.14137	0.309233	0.501687
SISC040	0.356086	0.566226	0.77432
SISC042	0.118811	0.253978	0.428405
WAMC002	0.309655	0.484122	0.663118
WAMC004	0	0.074297	0.266229

Sample	Lower limit 95% CI	Hybrid Index	Upper limit 95% CI
WAMC014	0.106802	0.292092	0.503433
WAMC031	0	0.051036	0.251256
WAMC036	0.041312	0.196237	0.400658
WAMC040	0	0	0.155469
WAMC054	0.034827	0.167643	0.364797
WAMC057	0.067241	0.244578	0.458567
WAMC063	0.101163	0.236751	0.416411
WAMC082	0	0	0.187056
WAMC084	0	0	0.107154
WAMC092	0	0.021512	0.154889
WAMC103	0.114417	0.260649	0.444939
WAMC114	0	0	0.116897
WAMC121	0	0	0.089098
WAMC127	0	0.025753	0.231995
WAMC128	0.009584	0.110593	0.303656
WAMC141	0.066132	0.174396	0.325955
WAMC148	0	0	0.09558
152G001	0.891268	1	1
152G002	0.582556	0.784241	0.942123
152G012	0.74665	0.940946	1
152G015	0.859403	1	1
152G018	0.814837	1	1
152G034	0.579304	0.803845	0.977303
152G035	0.585799	0.797395	0.960696
152G037	0.724972	0.952753	1
152G038	0.826736	1	1
152G040	0.882449	1	1
152G066	0.869392	1	1
152G068	0.385795	0.602523	0.818626
152G075	0.727981	0.960235	1
152G086	0.478255	0.7009	0.924138
152G093	0.931993	1	1
152G096	0.874091	1	1
152G098	0.852013	1	1
152G109	0.675388	0.912916	1
152G116	0.593441	0.816723	1
152G164	0.920002	1	1
152G199	0.929503	1	1
BOBG001	0.922129	1	1
BOBG002	0.801706	0.996334	1
BOBG003	0.830424	1	1
BOBG005	0.911736	1	1

Sample	Lower limit 95% CI	Hybrid Index	Upper limit 95% CI
BOBG007	0.582248	0.797163	0.964302
BOBG010	0.887584	1	1
BOBG034	0.923174	1	1
BOBG045	0.584187	0.772771	0.922413
BOBG057	0.543372	0.758394	0.956629
BOBG090	0.436498	0.653349	0.860381
BOBG094	0.556715	0.788343	0.99544
BOBG095	0.606299	0.810092	0.987354
BOBG104	0.599669	0.799819	0.95804
BOBG111	0.568403	0.791454	0.989195
BOBG114	0.492276	0.734013	0.959707
BOBG120	0.78981	0.992946	1
BOBG128	0.808458	1	1
BOBG158	0.718429	0.90766	0.993715
BOBG169	0.59672	0.817267	0.981772
BOBG174	0.818717	0.995739	1
BOBG198	0.87337	1	1
CATG013	0.563452	0.773858	0.954803
CATG038	0.620056	0.821377	0.969613
CATG073	0.510752	0.732792	0.927453
CHEG033	0.894356	1	1
CHEG048	0.838917	1	1
CHEG049	0.921617	1	1
CHEG088	0.861569	1	1
CHEG095	0.59017	0.813286	0.982705
CHEG096	0.521086	0.733629	0.91617
CHEG107	0.841466	0.971499	1
CHEG109	0.859295	0.990282	1
CHEG114	0.707317	0.900714	1
CHEG122	0.69541	0.933633	1
CRGG002	0.874063	1	1
CRGG008	0.540341	0.743466	0.922935
CRGG014	0.695741	0.892924	1
GDSG012	0.865858	1	1
HUNG002	0.547733	0.735811	0.888825
SISG003	0.829556	1	1
SISG011	0.635561	0.845318	1
SISG032	0.906714	1	1
SISG048	0.751961	0.915656	1
SISG050	0.355736	0.569317	0.787949
SISG066	0.910902	1	1
SISG067	0.905519	1	1

Sample	Lower limit 95% CI	Hybrid Index	Upper limit 95% CI
SISG069	0.828042	0.964182	1
SISG076	0.653263	0.899851	1
WAMG001	0.690165	0.908518	1
WAMG005	0.690977	0.876161	0.998092
WAMG008	0.825496	1	1
WAMG012	0.854275	1	1
WAMG016	0.695376	0.934704	1
WAMG020	0.793706	0.995487	1
WAMG031	0.482638	0.695667	0.885341
WAMG037	0.494283	0.702429	0.886004
WAMG038	0.638329	0.869924	1
WAMG040	0.860028	1	1
WAMG043	0.827625	1	1
WAMG050	0.707186	0.946399	1
WAMG055	0.72975	0.925781	1
WAMG062	0.462724	0.67395	0.88104
WAMG068	0.824537	1	1
WAMG075	0.917742	1	1
WAMG092	0.773307	0.932029	0.995833
WAMG096	0.852588	1	1

APPENDIX G: Estimated hybrid indices of plants

Hybrid index calculated using package INTROGRESS using method described in Buerkle (2005). Flies with a 0.99 or higher membership in a parental type using NEWHYBRIDS used as training samples. Values of 0 correspond to *I. coriacea* and 1 to *I. glabra*.

	Lower limit 95% CI	Hybrid Index	Upper limit 95% CI
P152C012	0	0	0.025698
P152C013	0	0	0.053315
P152C014	0	0.010338	0.088731
P152C031	0	0	0.043222
P152C042	0	0	0.040316
P152C077	0	0	0.033806
P152C092	0	0	0.036663
P152C099	0	0	0.038073
P152C122	0	0.004466	0.052682
P152C127	0	0	0.043644
P152C130	0	0	0.025685
P152C133	0	0	0.027589
P152C222	0	0	0.0296
P152C234	0	0	0.036493
P152C240	0	0	0.028275
P152C247	0	0	0.025766
P152C254	0	0.01984	0.08644
P152C271	0	0.042124	0.12622
P152C272	0	0	0.023759
P152C280	0	0.005776	0.057586
P152C288	0.084275	0.191218	0.312071
P152CE02	0.126972	0.219771	0.326941
P152CE06	0	0.000124	0.043281
PBOBC006	0	0	0.028814
PBOBC016	0	0	0.072876
PBOBC046	0	0	0.027086
PBOBC047	0	0	0.051491
PBOBC048	0	0	0.035279
PBOBC061	0	0	0.09251
PBOBC084	0	0	0.039025
PBOBC092	0	0	0.023988
PBOBC142	0	0	0.027247
PBOBC149	0	0	0.027793

	Lower limit 95% CI	Hybrid Index	Upper limit 95% CI
PBOBC181	0	0.035257	0.110599
PBOBC187	0	0.011242	0.068153
PBOBC191	0.085477	0.169217	0.269075
PBOBC198	0	0	0.026275
PBOBC228	0	0	0.034426
PBOBC240	0	0	0.026256
PBOBCE04	0	0	0.040278
PBOBCE05	0.335557	0.450678	0.569088
PCATC093	0.010444	0.120739	0.244897
PCATC115	0.176288	0.275032	0.383824
PCATC185	0	0	0.047636
PCATC197	0	0.002171	0.073857
PCATC204	0	0	0.037701
PCATC212	0	0.01814	0.088144
PCATC219	0	0	0.033301
PCATC225	0	0	0.04053
PCATC240	0	0.060716	0.15936
PCATC245	0	0.023525	0.120159
PGDSC003	0	0.001707	0.065924
PGDSC009	0	0	0.029495
PGDSC012	0	0.001237	0.061215
PGDSC024	0	0.031956	0.115365
PGDSC036	0	0.010401	0.079618
PGDSC055	0	0	0.038927
PGDSC057	0	0	0.043521
PGDSCE01	0	0.005507	0.057589
PGDSCE02	0	0	0.04712
PHUNC001	0	0.02963	0.125045
PHUNC003	0	0	0.054668
PHUNC006	0	0.048122	0.143082
PHUNC010	0	0.006221	0.090855
PHUNC012	0.194598	0.301103	0.414193
PHUNC014	0	0	0.067559
PHUNCE04	0	0	0.040135
PHUNCE06	0	0.006985	0.088297
PHUNCE08	0	0	0.045128
PSISC001	0	0	0.050524
PSISC009	0.063424	0.159493	0.271938

	Lower limit 95% CI	Hybrid Index	Upper limit 95% CI
PSISC010	0	0	0.053521
PSISC013	0	0	0.079916
PSISC025	0	0	0.065409
PSISC026	0	0	0.065497
PSISC028	0	0.084127	0.192921
PSISC033	0	0.007095	0.087982
PSISCE36	0.024638	0.108613	0.215233
PSISCE37	0	0.01682	0.118496
PSOPC001	0	0.009944	0.088487
PSOPC005	0.254694	0.360351	0.470751
PSOPCE01	0	0	0.057405
PSOPCE02	0	0.06609	0.1633
PSOPCE03	0	0	0.075683
PWAMC013	0	0	0.048819
PWAMC014	0	0	0.081011
PWAMC034	0	0	0.030449
PWAMC036	0	0	0.05216
PWAMC040	0	0.035807	0.118286
PWAMC046	0	0	0.032636
PWAMC057	0	0.020926	0.107268
PWAMC063	0	0	0.02808
PWAMC084	0	0	0.062606
PWAMC090	0.19029	0.292752	0.403628
PWAMC106	0	0	0.039684
PWAMC113	0	0	0.043323
PWAMC121	0	0.035198	0.107299
PWAMC123	0	0	0.042134
PWAMC128	0	0	0.065964
PWAMC141	0	0	0.028303
PWAMC144	0	0	0.025341
PWAMC148	0	0	0.024998
PWAMCE04	0	0	0.044783
PWAMCE07	0	0.015259	0.086885
P152G027	0.94886	1	1
P152G167	0.93114	0.990836	1
P152G168	0.971648	1	1
P152G172	0.944232	1	1
P152G174	0.957031	1	1

	Lower limit 95% CI	Hybrid Index	Upper limit 95% CI
P152G180	0.944899	1	1
P152G183	0.963427	1	1
P152G199	0.951566	1	1
P152GE01	0.966544	1	1
P152GE02	0.957848	1	1
PBOBG011	0.823666	0.931449	1
PBOBG028	0.933788	0.99176	1
PBOBG067	0	0	0.047665
PBOBG159	0.962983	1	1
PBOBG169	0.908178	0.98034	1
PBOBG170	0.957779	1	1
PBOBG174	0.920248	1	1
PBOBG182	0.909803	0.988887	1
PBOBG190	0.970665	1	1
PBOBG198	0.942747	1	1
PBOBG205	0.939187	0.996527	1
PBOBGE01	0.967177	1	1
PBOBGE17	0.956403	1	1
PCATG107	0.967214	1	1
PCATG114	0.963714	1	1
PCATG134	0.934101	0.993083	1
PCATG137	0.93544	1	1
PCATG143	0.960553	1	1
PCATG148	0.952656	1	1
PCATG151	0.964708	1	1
PCATGE09	0.950092	1	1
PCATGE17	0.9382	1	1
PCHEG003	0.955845	1	1
PCHEG033	0.959381	1	1
PCHEG047	0.933861	0.993413	1
PCHEG058	0.97114	1	1
PCHEG089	0.865035	0.955808	1
PCHEG093	0.955865	1	1
PCHEG114	0.955244	1	1
PCHEG119	0.962303	1	1
PCRGG001	0.89625	0.971309	1
PCRGG007	0.932682	1	1
PCRGG008	0.970856	1	1

	Lower limit 95% CI	Hybrid Index	Upper limit 95% CI
PCRGG010	0.964625	1	1
PCRGG013	0.940418	1	1
PCRGG014	0.893681	0.968683	1
PCRGG017	0.904673	0.979712	1
PCRGGE24	0.960689	1	1
PCRGGE32	0.968973	1	1
PCRGGE36	0.970409	1	1
PEAVG001	0.966078	1	1
PEAVG002	0.96704	1	1
PEAVG004	0.833854	0.931418	0.996201
PEAVGE02	0.925534	0.988188	1
PEAVGE03	0.958	1	1
PEAVGE06	0.951692	1	1
PEAVH003	0.838434	0.932911	0.995173
PEAVH004	0.964382	1	1
PEAVHE01	0.944697	1	1
PEAVHE02	0.953552	1	1
PGDSG018	0.849092	0.940017	0.993682
PGDSG020	0.943189	1	1
PGDSG021	0.880159	0.958813	1
PGDSG026	0.925581	1	1
PGDSG032	0.894918	0.979268	1
PGDSG037	0.956336	1	1
PGDSG046	0.888733	0.973004	1
PGDSGE05	0.844617	0.937768	0.998009
PGDSGE12	0.967166	1	1
PHUNG002	0.95316	1	1
PHUNGE01	0.912115	0.976066	1
PHUNGE05	0.401698	0.516045	0.630666
PHUNGE07	0.943692	1	1
PHUNGE09	0.958852	1	1
PHUNGE11	0.925399	1	1
PHUNGE14	0.952761	1	1
PHUNGE15	0.961249	1	1
PSISG006	0.95581	1	1
PSISG010	0.943579	1	1
PSISG032	0.824217	0.925274	0.998471
PSISG048	0.965583	1	1

	Lower limit 95% CI	Hybrid Index	Upper limit 95% CI
PSISG057	0.880786	0.968779	1
PSISG063	0.918511	1	1
PSISG076	0.953857	1	1
PSISGE16	0.959009	1	1
PSOPGE02	0.968661	1	1
PWAMG011	0.719095	0.817707	0.900909
PWAMG079	0.905637	0.975597	1
PWAMG091	0.962003	1	1
PWAMG093	0.959671	1	1
PWAMG094	0.93032	1	1
PWAMG096	0.963477	1	1
PWAMG097	0.945043	1	1
PWAMG098	0.972086	1	1
PWAMGE02	0.921292	0.988506	1
PWAMGE08	0.969113	1	1
PWAMGE15	0.867357	0.952031	1
PWAMGE19	0.956112	1	1

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