

ABSTRACT

Title of Document: SOLDIER NEOTENICS OF *ZOOTERMOPSIS NEVADENSIS* (HAGEN) AND *ARCHOTERMOPSIS* SP. (ISOPTERA: TERMOPSIDAE): MORPHOLOGY, DEVELOPMENT, BEHAVIOR, AND EVOLUTION

Susan E. Johnson, Master of Science, 2009

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I: The relictual reproductive soldier neoténics of primitive Termopsid termites may offer insights into the evolution of eusociality and sterile castes in termites.

II: Soldiers and helpers in a new Thai species of *Archotermopsis* do not display unusually complete gonad development. The first neoténic pair ever reported for this genus is described.

III: Morphological differences between soldiers and soldier neoténics in the head and gonads are quantified in *Z. nevadensis*. There is not necessarily a clear delineation in morphology between soldiers and soldier neoténics.

IV: Colony age and size do not significantly influence the differentiation or survival of male replacement reproductives in colonies of *Z. nevadensis*. Soldier neoténics' development is not significantly influenced by the presence of normal soldiers.

V: Soldier neoténics and related female neoténics tend to have greater initial survival after interactions with colonies with only normal neoténics. Mostly helpers are aggressors, never soldier neoténics or neoténics.

SOLDIER NEOTENICS OF *ZOOTERMOPSIS NEVADENSIS* (HAGEN) AND
ARCHOTERMOPSIS SP. (ISOPTERA: TERMOPTIDAE): MORPHOLOGY,
DEVELOPMENT, BEHAVIOR, AND EVOLUTION

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Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2009

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Chapter 1: Introduction and Overview

Termites (order Isoptera) are xylophagous insects, monophyletic with roaches (Inward et al., 2007; Legendre et al., 2008) that first appeared in the fossil record during the Cretaceous 130 million years ago (reviewed by Thorne et al., 2000). The order includes approximately 2,650 species (Abe & Higashi, 2001), all of which are eusocial.

The Challenge of Eusociality

The classic definition of eusocial behavior requires reproductive division of labor, overlapping generations, and cooperative brood care (Wilson, 1971), though it may also be seen as one end of a continuum of varying levels of reproductive skew and lifetime reproductive fitness (Sherman et al., 1995). Eusociality can be a challenge to understand in a traditional Darwinian context, in that most colony members forgo reproduction and behave altruistically throughout their lives. Much discussion is still occurring regarding the mechanisms behind the evolution of eusociality (e.g. kin selection vs. group selection: Wilson, 2005; Hölldobler & Wilson, 2005; Foster et al., 2005). A number of factors have been implicated in the evolution and maintenance of eusociality; both genetics and ecological dynamics seem to be important, though in varying degree among taxa.

The eusocial Hymenoptera have been especially well-studied. They contain genetic relatedness asymmetries which cause female workers to be more closely related to

sisters than to offspring, thus providing a means for them to remain sterile yet care for their sisters and ensure that their genes—contained in those sisters—are passed on (Hamilton, 1964). Although a number of contributing factors are probably important to the evolution of eusociality in the Hymenoptera, including ecological effects (reviewed by Bourke & Franks, 1995 and Foster et al., 2005; Wheeler et al., 2006), indirect fitness resulting from genetic relatedness asymmetries has classically been an important factor explaining eusociality in this order, as well as a predictor of intracolony behaviors (Trivers & Hare, 1976; Charnov, 1978), though relatedness asymmetries do not exist in some extant taxa (e.g. Strassmann et al., 1989).

Termites and Eusociality

Unlike the Hymenoptera, the Isoptera are diploid and do not seem to possess relatedness asymmetries between sexes or castes (reviewed in Husseneder et al., 1999). There are several other diploid eusocial taxa, including several species of snapping shrimp (Duffy, 1996), naked mole rats (Jarvis, 1981), a species of ambrosia beetle (Kent & Simpson, 1992), and some aphids (reviewed by Pike & Foster, 2008), that also do not fit into the classic model of indirect fitness and kin selection as applied to the Hymenoptera. While relatedness within a colony of termites is a likely contributor to social behavior, differential relatedness among individuals is not nearly as important among the termites as it is among the social Hymenoptera (e.g. Nalepa, 1994; Thorne, 1997; Shellman-Reeve, 1997a; Thorne et al., 2003; Korb, 2008; Korb & Heinze, 2008).

Termites, like ants, evolved eusociality once ancestrally, with no subsequent losses in any taxa, which has been confirmed by recent molecular phylogenies (Moreau et al., 2006; Inward et al., 2007). However, other Hymenoptera, such as halictid bees and vespid wasps, contain both social and non-social taxa, reflecting their evolutionary history of multiple gains (and sometimes losses) of the social state (Cameron, 1993; Schmitz & Moritz, 1998; Danforth et al., 2003; Brady et al., 2006). This allows study of the conditions under which eusociality might be beneficial to a species and could evolve. In the Isoptera lack these extant “stepping stones” of species from solitary to eusocial, making it far more difficult to study the transitions (reviewed in Thorne, 1997; Shellman-Reeve, 1997). Instead, the most behaviorally primitive of the eusocial termites may provide clues to the evolution of eusociality in this clade (Thorne, 1997; Thorne et al., 2000; Thorne & Traniello, 2003).

According to the comprehensive molecular and morphological phylogeny of Inward *et al.* (2007), the most basal clades of termites are *Mastotermes* followed by a sister group of the Termopsids *Zootermopsis* and *Archotermopsis*. A more recent phylogeny, which uses only genetic information, differs from Inward *et al.* in its placement of the Termopsids (Legendre et al., 2008). This phylogeny retains Mastotermitidae as the most basal termite family but places Kalotermitidae as the next-derived family, with Termopsid and Hodotermpsoid genera as sister groups. The exact placement of these primitive families has long been disputed (reviewed by Kambhampati & Eggleton, 2000); though *Mastotermes darwiniensis* has long been accepted as the most primitive living termite in both of these recent phylogenies as

well as all previous phylogenies (e.g. Wheeler, 1904; Snyder, 1926; Kambhampati et al., 1996; Kambhampati & Eggleton, 2000; Thompson et al., 2000). Although *Mastotermes* possesses a primitive, cockroach-like morphology (Desneux, 1904), it also has several highly derived characteristics (reviewed by Thorne & Carpenter, 1992), such as large nest structures with gallery construction, extensive subterranean foraging tunnels, and very large colony populations (Emerson, 1938), making it unsuitable as a model for the study of the ancestral conditions of termite life. In contrast, the Termopsids *Zootermopsis* and *Archotermopsis* are behaviorally and developmentally primitive. Termopsids are “one-piece nesters,” meaning they consume only the piece of wood in which they live (Abe, 1987). They live in relatively small colonies inside their food, with the “worker” role filled by developmentally and behaviorally flexible “helpers” rather than a terminal-stage true worker caste (Imms, 1919; Noirot & Pasteels, 1987; Rosengaus & Traniello, 1993; Thorne & Traniello, 2003). Developmental flexibility is required with a one-piece nesting life-history because the food will eventually run out and developmental multipotency allows many of the termites in the nest the opportunity to moult into winged alates to disperse (Abe, 1987; Higashi et al., 1991; Shellman-Reeve, 1997a). Because these life-history traits are ancestral (Inward et al., 2007), Termopsids such as *Zootermopsis* and *Archotermopsis* are useful models for investigating eusocial evolution in termites (Thorne, 1997; Thorne & Traniello, 2003).

Moving Beyond Hamilton's Rule: Ecology and Eusociality

Because termites lack clear relatedness asymmetries, research has instead suggested a set of ecological and life history factors as the driving force in termite social evolution (e.g. Alexander et al., 1991; Nalepa, 1994; Shellman-Reeve, 1994; Shellman-Reeve, 1997a; Thorne, 1997), that include:

1. **Diet.** Termites feed on cellulose, (primarily wood), a nitrogen-poor material that is difficult to digest without the aid of cellulotic symbionts in the midgut (Waller & LaFage 1987). Because newly eclosed termites have no symbionts, they must receive them from an older individual. Based on study of the subsocial sister group to termites (Inward et al., 2007), the cockroach *Cryptocercus*, microorganism transfer has been hypothesized to be one of the forces driving the initial evolution of group living in the ancestor to termites (Cleveland et al., 1934; Nalepa, 1988; Nalepa, 1994; Nalepa & Bandi, 2000). However, some wood-feeding cockroaches do not require prolonged group contact to transfer symbionts (Shellman-Reeve, 1997a; Pellens et al., 2007) and significant life-history differences between *Cryptocercus* and termites may limit inferences about termite evolution based on this roach (Thorne, 1997; but see Klass et al., 2008).

Because termites' diet is nutrient-poor, they develop very slowly (Nalepa, 1988; Nalepa, 1994; Shellman-Reeve, 1997a; Thorne, 1997; Roux & Korb, 2004). This slow development was probably an influence on eusocial evolution in termites (Nalepa, 1988; Alexander et al., 1991; Nalepa, 1994; Shellman-Reeve, 1997a;

Thorne, 1997; Roux & Korb, 2004), as well as in the eusocial Curculionid ambrosia beetle (Kent & Simpson, 1992) because the longer period of maturation for juveniles due to a poor diet requires both parents' presence for survival (Nalepa & Jones, 1991; Shellman-Reeve, 1994; Shellman-Reeve, 1997b).

2. Disease immunity. Some species of termites, when isolated, have significantly lowered immune responses than termites living in groups (Rosengaus et al., 1998; Traniello et al., 2002). Oocyte production by the reproductive female also decreases her immune response (Calleri et al., 2007). Disease resistance through allogrooming may, then, have been a factor leading to group living in the evolution of termites (Rosengaus et al., 1998; Traniello et al., 2002; Callieri et al., 2007).

3. Defense. Termite nests are highly secure and are valuable resources to their inhabitants. Group defense of such “fortresses” imparts a significant benefit to all individuals in the colony at a low average cost, and may be key in the evolution of sociality in all taxa that possess them (Nutting, 1969; Alexander et al., 1991; Stacey & Ligon, 1991; Kent & Simpson, 1992; Nalepa, 1994; Duffy, 1996; Shellman-Reeve, 1997a; Thorne, 1997; Roux & Korb, 2004; Chapman et al., 2008; Korb, 2008; Pike & Foster, 2008). Observation of natural *Z. nevadensis* colonies shows that biparental defense of the nest-fortress is key to offspring survival in young colonies (Shellman-Reeve, 1997b).

4. **High cost of dispersal.** The winged dispersing forms, called alates, suffer high mortality upon leaving the nest and founding a new colony (reviewed by Nutting, 1969). A high risk of dispersal away from the nest-fortress may have contributed to the evolution of non-dispersive castes that stay in the nest as long as possible (Alexander et al., 1991; Nalepa & Jones, 1991; Thorne, 1997; Roux & Korb, 2004; Korb & Heinze, 2008) until encouraged to moult into alates by limited food conditions (Shellman-Reeve, 1997a; Korb & Katrantzis, 2004; Korb & Schmidinger, 2004).

5. **Inheritance.** Because these fortress-nests are valuable and limited in number (reviewed by Korb & Heinze, 2008), they provide an incentive for individuals to postpone dispersal and stay in the nest in the hopes of inheriting it upon death or senescence of reproductive individuals (Wilson, 1971; Alexander et al., 1991; Thorne, 1997; Thorne et al., 2003; Thorne & Traniello, 2003; Korb, 2008). Termites' slow, flexible development allow them to “bide their time” waiting for a chance at inheritance (Thorne, 1997; Roux & Korb, 2004). High intraspecific competition between colonies, resulting in early death of reproductive individuals, may aid in chances of nest inheritance (Thorne et al., 2002; Thorne et al., 2003; Korb, 2008). Experimental manipulation of primitive Kalotermitid *Kaloterme*s colonies in order to increase opportunities for indirect fitness did not decrease alate production, suggesting that nest inheritance was more important to “helper” termites than indirect fitness (Korb, 2007).

The ecological factors listed above may provide the strongest causative and sustaining forces in the evolution and maintenance of eusociality and altruistic castes in termites (e.g. Alexander et al., 1991; Nalepa, 1994; Shellman-Reeve, 1994; Shellman-Reeve, 1997a; Thorne, 1997; Thorne & Traniello, 2003; Korb, 2008). In my research, I focus on ecological and life-history factors' influence on the origin of the altruistic and reproductive castes in *Zootermopsis* and *Archotermopsis*.

A Brief Introduction to a Termopsid Colony

Colonies of the primitive Termopsids *Archotermopsis* and *Zootermopsis* are founded by two primary reproductives (the king and queen), which are derived from alates, the winged dispersal form. Upon pairing under the bark of a dead tree, the young king and queen drop their wings and never leave the colony again (reviewed by Nutting, 1969).

The youngest of the colony's members are the larvae. The use of the term "larvae" to denote the youngest juveniles in the colony is formally a misnomer since termites are hemimetabolous and all immatures are technically nymphs. However, it is convention to use the term "larvae" for instars I-III. In termites, the term "nymph" has a narrower definition, referring only to those individuals with developing wings (reviewed in Thorne, 1996).

During the fourth instar and beyond, unspecialized juveniles are termed "helpers." The term 'helper' is used in the lower termites instead of "worker" because there is

no endpoint-stage worker caste in these termites, and it avoids confusion with true worker castes in higher termites and the Hymenoptera (reviewed in Thorne, 1996). Helpers maintain a developmental flexibility that allows them to progress into reproductive forms or undergo stationary moults (Noirot & Pasteels, 1987) depending on colony cues.

Older colonies also produce alates—winged dispersers and founders of new colonies—but these usually leave quickly rather than staying and contributing to the reproduction inside their natal nest (reviewed by Nutting, 1969 and Stuart, 1969). Alates develop through brachypterous, or “nymphal” stages (reviewed by Brian, 1957).

Soldiers are a defensive caste in a termite colony (reviewed by Noirot, 1969) found in all termites, except in the Termitid subfamily Apicotermitinae, in which they have been secondarily lost (Inward et al., 2007). Their large, sclerotized heads and hypertrophied mandibles are specialized for defense (reviewed by Weesner, 1969a), though helpers and workers may also be aggressive defenders (e.g. Nel, 1968, Thorne, 1982, Haverty and Thorne, 1989, Polizzi and Forschler, 1998, Delphia et al., 2003). In some termites, soldiers may have alternative roles, such as foraging recruitment (Traniello, 1981). Like reproductives, soldiers are “terminal” forms that cannot moult into another caste (reviewed by Roisin, 2000) despite their retention of the prothoracic glands that produce molting hormones (Noirot & Pasteels, 1987).

Implications of Developmental Plasticity for Reproduction in Termopsids

Termopsids' unique developmental plasticity allows helpers and nymphs to moult into neotenics under certain circumstances (e.g. Castle, 1934a; Light & Weesner, 1951; Noirot, 1985; Noirot & Pasteels, 1987). Neotenics are reproductive forms that either replace a dead primary of the same sex or supplement the reproductive capabilities of large colonies' primaries. They rarely develop when the primary king or queen of the same sex is present (Light, 1943; Light & Weesner, 1951) due to inhibition of corpora allata activity (Greenberg & Tobe, 1985). Neotenics develop from helpers or nymphs through at least one, but generally two special moults (reviewed by Roisin, 2000). Development of neotenics from helpers is more common than nymph-derived neotenics (Light & Weesner, 1951). In *Z. nevadensis*, neotenics cannot develop from the first three instars; they usually develop from individuals in instars 7-10. Light and Weesner (1951) estimate that while most juveniles in a colony are potentially capable of reproduction, only 3% or fewer of the proper-age individuals are capable of developing into a neotenic at any time (Light & Weesner, 1951). Colonies may have multiple neotenics, often with more than one female, especially in larger colonies (reviewed by Roisin, 2000).

Normal neotenic development is fairly well-understood, and is mediated through JH control of the genome (Greenberg & Tobe, 1985; Elliott & Stay, 2007; Cornette et al., 2008; Elliott & Stay, 2008). There is disagreement in the literature as to whether neotenic production is triggered by high juvenile hormone (JH) production (Elliott & Stay, 2008) or low JH production (Cornette et al., 2008) though JH is known to vary

by season, food availability, and colony composition (reviewed by Korb & Hartfelder, 2008). Little is known about the genes upregulated in neotenic development, though it is known that cytochrome p450 genes of the cyp4 family are among these (Zhou et al., 2006b).

Although most neotenics are usually of the helper-derived (“normal”) form, in six species of primitive Termopsid termites, (Termopsinae: *Archotermopsis wroughtoni*, *Zootermopsis angusticollis*, *Zootermopsis nevadensis*, *Zootermopsis laticeps*; Stolotermitinae: *Stolotermes brunneicornis*, *Stolotermes ruficeps*), some neotenics may have soldier-like morphologies, and are called reproductive soldiers, or soldier neotenics (reviewed by Myles, 1986). Soldier neotenics, while possessing the generalized defensive soldier form, do generally have slight differences in external morphology that distinguish them from normal soldiers. These differences include a slightly rounder head shape and shorter, more curved mandibles (Heath, 1928; Castle, 1934b; Morgan, 1959; Myles, 1986), but distinctions have never been extensively or quantitatively examined. Additionally, in *Zootermopsis nevadensis* they exhibit behavior more similar to a neotenic than a soldier (Heath, 1928; Thorne et al., 2003). Because soldier neotenics occur only in socially and ecologically primitive termites, they are considered evolutionary relicts of an intermediate form of soldiers: a stepping-stone on the way to obligatory sterility and altruistic defense (Myles, 1986; Thorne et al., 2003, but see Roisin, 1999). However, little is known of soldier neotenics’ evolution, development, frequency within termite colonies, or behavioral

roles, despite their apparent importance in understanding the evolution of eusociality and soldiers in termites.

Soldiers and Soldier Neotenic

Soldiers occur in several other eusocial clades of insects, including thrips, aphids, and many ants (soldier forms in ants are called major workers, or majors). In these clades, soldiers do not generally reproduce. One lineage of thrips basal to the eusocial groups has fertile soldiers, which have a high direct fitness relative to other colony members and produce most of the colony's dispersers (Kranz et al., 2001). In these thrips, loss of wings (leading to soldier morphology) is regarded as a trade-off in order to gain the high fitness resulting from fertile disperser production (Kranz et al., 2001); thus these thrips are not directly analogous to the soldier neotenic of termites. In aphids, soldiers vary in number, aggression, and morphology depending on factors such as predation, population density, and colony reproductive rate (reviewed by Pike & Foster, 2008). They are always sterile, but gain substantial indirect fitness due to the clonal nature of aphid colonies. Clonality alone, however, is not sufficient for soldier maintenance, as soldiers were gained and lost many times throughout aphid evolution (Pike & Foster, 2008).

Ant soldiers are usually considered a type of worker (Wheeler & Nijhout, 1983; Bourke & Franks, 1995; Hughes et al., 2003; Fjerdingstad & Crozier, 2006; Hughes & Boomsma, 2007), though some recent work suggests they may be evolutionary derivatives of gynes (Urbani, et al., 1996). They tend to develop in species with early

caste differentiation (Fjerdingstad & Crozier, 2006), especially in larger colonies of these species (Bourke & Franks, 1995), and are necessary for colony survival and growth (Bourke & Franks, 1995). Although there is some genetic basis to the extent of soldier development in ants (Fraser et al., 2000; Hughes et al., 2003; Fjerdingstad & Crozier, 2006; but see Bourke & Franks, 1995), the ability of an ant larva to develop into a soldier seems to be a plastic response based on a critical period of sensitivity to exogenous JH (Wheeler & Nijhout, 1983; Hughes et al., 2003), the presence of mature soldiers (Wheeler & Nijhout, 1984), and environmental conditions (Hughes et al., 2003; Hughes & Boomsma, 2007).

In termites, normal soldier development is mediated by JH and hexamerin proteins, (Park & Raina, 2004; Zhou et al., 2006a; Zhou et al., 2007; Cornette et al., 2008; Elliott & Stay, 2008) that regulate soldier-specific genes (Scharf et al., 2003; Hojo et al., 2005) through mostly-unidentified signaling cascades (Cornette et al., 2006). Specifically, high JH titers lead to a moult into a soldier (Park & Raina, 2004; Cornette et al., 2008;), while high hexamerin concentrations have the opposite effect (Zhou et al., 2006a). However, only a few juveniles may be responsive at any time to hormonal inducing development into a soldier (Park & Raina, 2004). In lower termite families, such as the Termopsids, soldier development is more flexible than in higher termites, and soldiers may develop even from nymphs, however, the overall pattern of soldier development is conserved in all termites (Roisin, 2000). Development of new termite soldiers is inhibited by the presence of mature soldiers (Park & Raina, 2004; Zhou et al., 2006a) and the size of newly-developed soldiers is directly proportional

to the colony size (Crosland et al., 2006). Soldier caste determination is likely conserved, as the presence of soldiers is an ancestral trait that arose once, at the origin of the termites (Inward et al., 2007; Legendre et al., 2008).

The development and evolution of the Termopsid soldier neotenic is poorly understood. Heath (1928) and Castle (1934b) proposed that soldier neotenic derive from individuals developing toward a soldier morphology but that are diverted onto a reproductive pathway before the final molt into a soldier. Roisin (1999) has suggested that soldier neotenic morphology may be a side effect of JH's dual role as both a trigger for soldier development and a gonadotrophic hormone for reproductives. Application of juvenile hormone analog (JHA) on nymphs of *Z. nevadensis* induces an alate/soldier intercaste which displays gonad development and a morphology very similar to that of naturally-occurring soldier neotenic (Miura et al., 2003). However, no data exist to demonstrate if soldier neotenic naturally develop from nymphs (as in the laboratory exogenous JHA applications by Miura et al., 2003), or if they diverge from the developmental pathway of normal soldiers. Prior research has shown, however, that soldier neotenic arise frequently in colonies following intercolony interactions (Thorne et al., 2003).

Intercolony Interactions, Soldier Neotenic, and the Evolution of Eusociality

Battles between colonies often occur between incipient Termopsid colonies; many pairs of alates may colonize the same piece of wood, and as these growing colonies contact one another, meetings between neighboring colonies are inevitable (Thorne et

al., 2002). If reared in isolated colonies, the founding primaries may live for many years (reviewed by Thorne et al., 2002), usually repressing reproduction by other colony members (reviewed in Miller, 1969). As discussed previously, the chance of inheriting the nest was probably a very strong driving force in the social evolution of termites and other fortress-building animals. However, longevity of primary reproductives would seem to drastically limit inheritance opportunities (Roisin, 1999), making staying and helping a less-favorable option to young colony members. Frequent intraspecific conflict, however, significantly shortens primary reproductives' lifespans (Thorne et al., 2002; Thorne et al., 2003). The Accelerated Inheritance Hypothesis states that the common occurrence of interactions between growing termite families sharing the same piece of wood—resulting in early death of primary reproductives—provides opportunities for helper offspring, even in very young colonies, to develop into reproductives (Thorne et al., 2003). Development of a soldier neotenic may increase a colony's chance of survival and victory in subsequent battles. Previous research has demonstrated that soldier neotenics are effective at assassinating opposing primary reproductives of the same sex in intercolony conflicts (Thorne et al., 2003). No data yet exist on aggressive behavior by soldier neotenics toward normal neotenics, a dynamic critical for analyzing the costs and benefits of the production of each in the context of intercolony conflicts. Understanding the social and ecological conditions under which each type of neotenic reproductive develops, especially the unusual and apparently primitive soldier neotenics, will illuminate selective pressures driving sterile and reproductive developmental pathways. The “higher termites” (Termitidae) possess true worker castes with limited options to

moult into neotenics and they lack reproductive soldier forms. Thus, the more primitive termites maintain a reproductively flexible state that must have been present as an intermediate form between non-social pre-termites and the rigidly eusocial higher termites that exist today (e.g. Noirot, 1985; Noirot & Pasteels, 1987; Abe, 1987; Thorne, 1997; Thorne & Traniello, 2003). Understanding the development of the Termopsids' soldier neotenics may offer further insights into the evolution of eusocial castes in termites.

My research focuses on the following questions: Do soldier neotenics exist in a new species of *Archotermopsis* recently discovered in Thailand? What are the morphological differences, especially diagnostic differences, between soldier neotenics and normal soldiers? Which colony circumstances lead to the differentiation of soldier neotenics? What are the differences in behavior and survivorship between soldier neotenics and normal neotenics in intraspecific intercolony conflicts?

Chapter 2: *Archotermopsis* sp. (Termopsidae) of Northern Thailand: Gonad Development in Soldiers and Description of Neotenic Pair, with General Observations on Life-History

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Abstract

Two-hundred eighty-four termites identified as the rare, relictual genus *Archotermopsis* (Termopsidae) were collected from one fallen log of *Toona ciliate* in a mixed deciduous forest at approximately 1500 m elevation in Doi Phu Ka National Park, Nan Province, Thailand in February of 2008. Among them was the first neotenic pair ever recorded for this genus. The neotenics exhibit much greater gonad development than the other individuals collected with them. Gonad development of neotenics and helpers is similar to that in the closely-related species *Zootermopsis nevadensis* (Hagen), as is mandible development in the neotenics. These results suggest that not all individuals of the genus *Archotermopsis* have fully-developed gonads, as was recorded in Imms' 1919 monograph on *Archotermopsis wroughtoni*.

In February 2008 we visited Doi Phu Kha National Park (19°36'N, 100°54'E), Thailand, and surveyed sites for the rare termite genus *Archotermopsis*. Doi Phu Kha Park consists of 1,704 km² of mountainous terrain (300-1980m asl) in northern

Thailand's Nan Province. Ninety percent of the site is covered with mixed deciduous forest, remaining areas include pockets of hill evergreen forest, dry evergreen forest and deciduous dipterocarp forest (Pimathai et al., 2004). During several days of searching, only one log inhabited by *Archotermopsis* sp. was found. This was a wet, rotting log of *Toona ciliate* (family Meliaceae), about 50 cm in diameter and 8 m long. It was the same log from which the species was originally collected in 2003, for the first and only record from Thailand (Sornnuwat et al., 2004a, 2004b; Vongkaluang et al., 2005; Chutibhapakorn & Vongkaluang, 2006). A Japanese team involving Osamu Kitade, Tadao Matsumoto, and Yoko Takematsu are working on this species in comparison to other species found in Southeast Asia (personal communication). Although Kitade, Matsumoto, and Takematsu have preliminarily assigned it to the genus *Archotermopsis*, its taxonomic assignment and description have yet to be fully resolved.

Archotermopsis wroughtoni (Desneaux) from the Himalayan foothills has been described only from coniferous forests, inhabiting fallen softwood logs and stumps (Imms, 1919; Roonwal et al., 1984), whereas *A. kutznetzovi* (Belyaeva) from Vietnam occurs in humid tropical forest (Belyaeva, 2004). The log we sampled was located in a moist clearing of a mixed deciduous forest at approximately 1500m elevation, with a rivulet of flowing water close to the log. There were no signs of termite habitation on the exterior of the log. The site was northeast of the main park headquarters, approximately 2-3 km along the 'Natural Study Trail,' and about 300 m below the trail. We sampled the log on two separate days. A total of 55 brachypterous nymphs,

177 late-instar apterous "workers"¹, 15 soldiers, 35 larvae², and a pair of neotenic reproductives (Fig. 1) were collected. Sampling was not exhaustive to ensure survival of the colony/colonies, and only about 2m of log section were opened up along its top side.



Fig. 1. Live female and male neotenic pair of *Archotermopsis* sp. from Thailand.

¹ In primitive termites, there is no endpoint-stage worker caste. The individuals we refer to as "workers" are actually juveniles with slowed development. They are also referred to as "helpers" (Thorne, 1996) and as "worker-like forms" (Imms, 1919).

² The use of the term "larvae" to denote the youngest juveniles in the colony is formally a misnomer since termites are hemimetabolous and all immatures are technically nymphs. However, it is convention to use the term "larvae" for instars I-III. In termites, the term "nymph" has a narrower definition, referring only to those individuals with developing wings (reviewed in Thorne, 1996).

No primary reproductives were found. The neotenics, along with 6 soldiers, 8 "workers", 22 nymphs and all of the larvae, were found together in one small pocket in the log. It could not be determined if one or several colonies from this log were collected. To our knowledge, no neotenics from the genus *Archotermopsis* have yet been described (Imms, 1919; Roonwal, 1979; Roonwal et al., 1984; Belyaeva 2004). Thus, this report focuses on the neotenics, especially the female. Due to regulatory requirements pertaining to specimens collected in Thailand's national parks, most of the collected individuals, including the male neotenic, remain in Thailand for future study.

Several external and internal measurements of each of the neotenics were obtained (Table 1) using a micrometer eyepiece on a dissecting microscope. Both neotenics possessed darker heads than either the nymphs or the "workers" (Fig. 2). Of 24 individuals dissected (2 neotenics, 6 nymphs, 11 soldiers, and 6 "workers": 11 males, and 14 females—gender determined by shape of abdominal sternites), the male neotenic was the only male with apparent testes (Fig. 3) and the female neotenic was the only egg-producing female, with visible ovarioles and much larger ovaries (Table 2) than any other female individuals examined (Fig. 4).

	Male Neotenic (n=1)	Female Neotenic (n=1)
Total body length (not including antennae)	12.25 mm	11.0 mm
Head length (posterior margin to anterior margin of sclerotization of head capsule)	2.45 mm	2.33 mm
Head width	3.04 mm	2.6 mm
Eye length	0.53 mm	0.55 mm
Eye width	0.38 mm	0.35 mm
Ovary length (apex to base of most posterior ovariole)		3.26 mm
Ovary width at midpoint		0.74 mm
Ovary width at widest point		0.86 mm
Number of eggs		4 (2 per ovary)
Widest testis diameter	0.78 mm	
Narrower testis diameter	0.40 mm	

Table 1. Comparative morphometry of the male and female neotenic of

Archotermopsis sp.



Fig. 2. Head of the male neotenic of *Archotermopsis* sp.

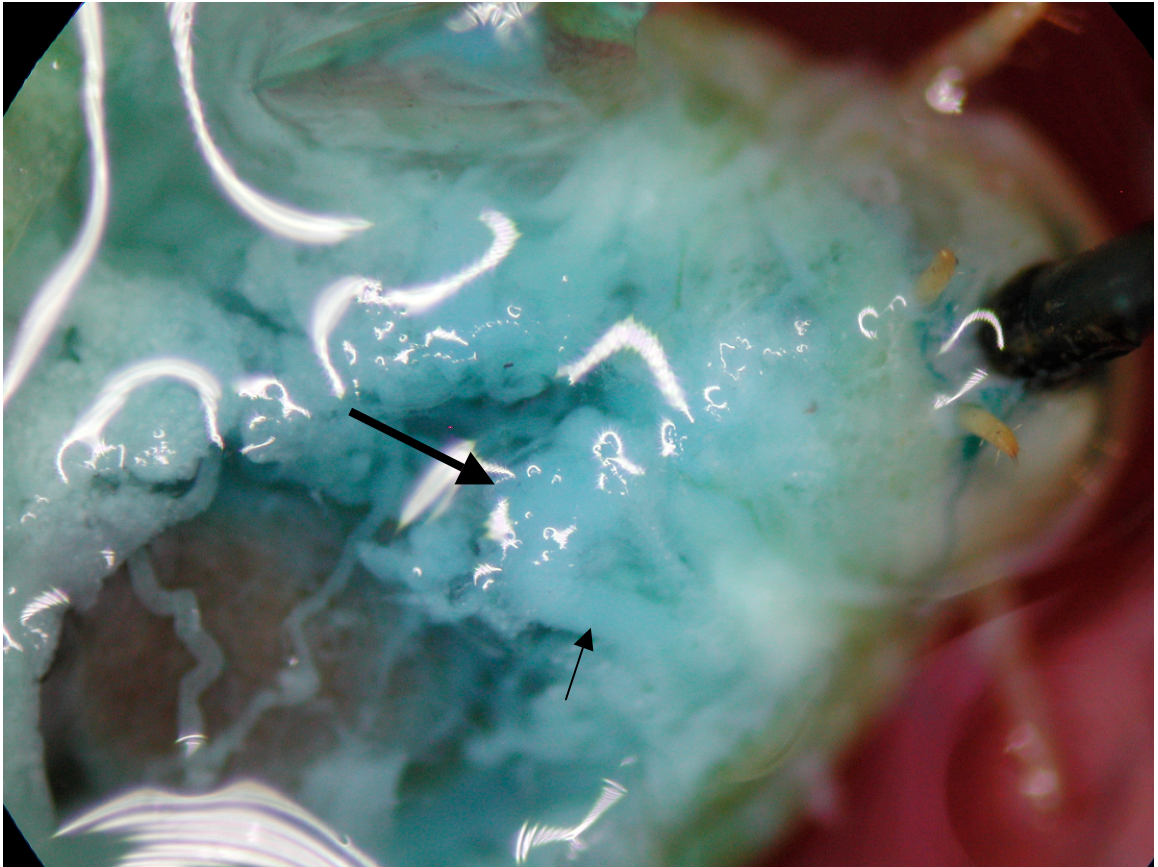


Fig. 3. Well-developed testis of neotenic male *Archotermopsis* sp. Thick arrow indicates testis, narrow arrow indicates vas deferens.

	Ovary length (apex to base of most posterior ovariole)	Ovary width at midpoint	Ovary width at widest point
Neotenic (1)	3.26 mm	.74 mm	0.86 mm
Nymphs (1) **	0.73	0.40	
“Workers” (3)	1.49±0.86	0.35±0.20	0.43±0.25
Soldiers (6) *	1.72±0.31	0.32±0.05	0.41±0.04

Table 2. Mean ovary size by caste in *Archotermopsis* sp., with standard errors of the

mean. (Number of individuals measured for each caste indicated in parentheses.

Each asterix signifies an additional dissected individual for whom ovaries were not located. These were not used to calculate the mean or standard errors.)

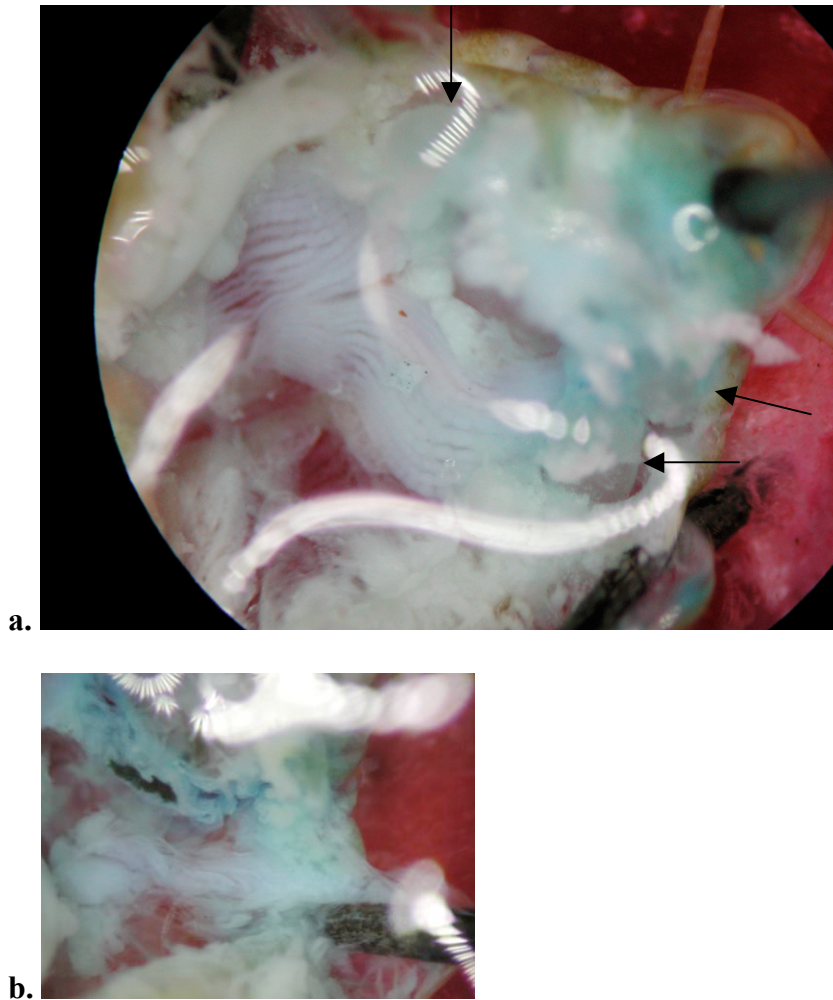


Fig. 4. Ovaries of neotenic (a) and "worker" (b) of *Archotermopsis* sp. Photos are not to scale; Ovary in (a) measured 3.26mm in length and 0.74mm width at midpoint; and in (b) 1.97mm in length and 0.43mm width at midpoint. Three eggs of the existing 4 are visible in (a); they are indicated by the black arrows.

Gonad development of females in the closely-related species *Zootermopsis nevadensis* (Hagen) is comparable to females of the Thai species of *Archotermopsis*. *Z. nevadensis* males are more likely to exhibit testis development than the Thai *Archotermopsis* (Table 3a,b), though this might be an artifact of the higher-powered

microscope used in the laboratory dissections of *Z. nevadensis* than in the field dissections of *Archotermopsis* sp. Due to the unknown age and population size of the field-collected *Archotermopsis*, it is impossible to determine if the *Z. nevadensis* individuals dissected for comparison were of a similar age, though effort was made to ensure they were of similar overall body size to each other.

	Ovary length (apex to base of most posterior ovariole)	Ovary width at midpoint	Ovary width at widest point
Nymphs (9)	1.81 mm ($\pm .08$)	.28 mm ($\pm .02$)	.36 mm ($\pm .02$)
“Workers” (13)	1.42 mm ($\pm .05$)	.26 mm ($\pm .008$)	.29 mm ($\pm .01$)
Soldiers (59)*	1.66 mm ($\pm .09$)	.21 mm ($\pm .02$)	.27 mm ($\pm .02$)

Table 3a. Mean ovary size by caste in *Zootermopsis nevadensis*, with standard errors

of the mean. (Number of individuals measured for each caste indicated in parentheses. Each asterix signifies an additional dissected individual for whom ovaries were not located. These were not used to calculate the mean or standard errors.) Nymphs and workers were taken from three laboratory stock colonies. Soldiers were taken from a wide variety of laboratory stock colonies.

	Widest testis diameter	Narrower testis diameter
Nymphs (18)***	.47 mm (\pm .03)	.39 mm (\pm .02)
“Workers” (13)****	.39 mm (\pm .02)	.33 mm (\pm .02)
Soldiers (74)	.43 mm (\pm .02)	.32 mm (\pm .01)

Table 3b. Mean testis size by caste in *Zootermopsis nevadensis*, with standard errors

of the mean. (Number of individuals measured for each caste indicated in parentheses. Each asterix signifies an additional dissected individual for whom testes were not located. These were not used to calculate the mean or standard errors.)

“Narrower testis diameter” was measured at ninety degrees from the widest testis diameter. Nymphs and workers were taken from three laboratory stock colonies.

Soldiers were taken from a wide variety of laboratory stock colonies.

Imms (1919) reported his extraordinary observation that “there is reason to believe...that [in *Archotermopsis wroughtoni*] the soldiers are not infrequently fertile, and it is likely that all the individuals of that caste are potentially capable of reproduction.” He also wrote that “all [seven “workers” he dissected] had well-developed gonads, the females containing several nearly full-sized eggs.” Neither the soldier nor “worker” material from Thailand showed such gonad development. Imms (1919) observed *A. wroughtoni* “workers” only in May and June and suggested their appearance was seasonal; however, the Thai species was found in February.

Mandibles of the Thai *Archotermopsis* sp. neotenics are of a similar size and dentition to those of “workers”, the only difference being coloration. The neotenics’ mandibles are completely dark and hardened (although slightly darker at the teeth and molar

plate), while the “workers” only have this more complete sclerotization along the teeth and molar plate (Fig. 5). The dentition patterns resemble those described previously for imagos of this genus (Belyaeva, 2004).

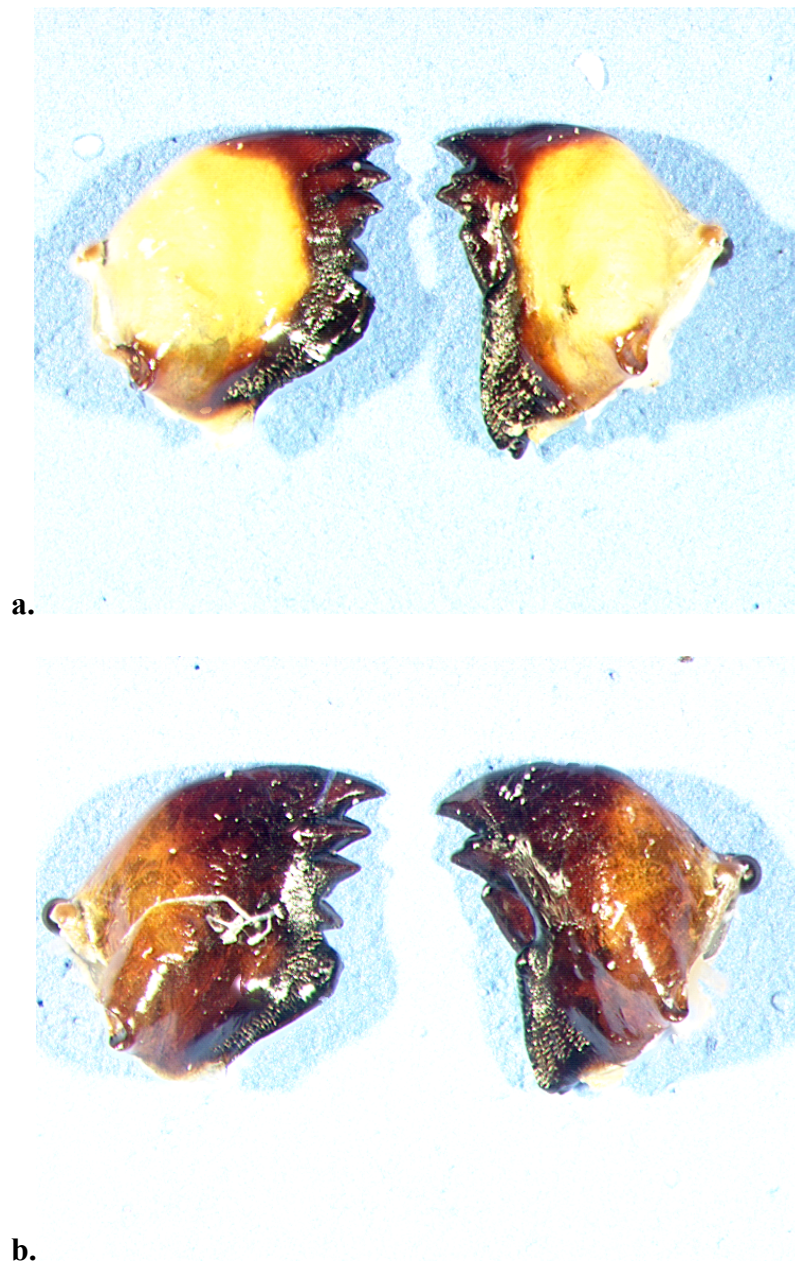


Figure 5. Mandibles of “worker” (a.) and female neotenic (b.) of *Archotermopsis* sp., at 32x magnification.

Summary

1. The first neotenic reproductives for termites of the genus *Archotermopsis* were discovered in Nan Province, Thailand in a still-unnamed species of the genus.
2. The two neotenics studied had much greater gonad development than "workers" and soldiers within the colony.
3. Various morphologic features of the neotenics are described, including head capsule and mandibles.
4. Unlike *A. wroughtoni*, soldiers and "workers" of the Thai species did not have fully-developed gonads.

Acknowledgements

We would like to acknowledge the Thai Royal Forest Department for their collaboration and assistance, as well as the staff of Doi Phu Ka National Park. Napalai Samerchai, Wasachol Pengyam, and Jakrapan Karnchanapa provided invaluable assistance in specimen collection and logistics. Boonsom Ubolwanee provided transportation between Bangkok and Doi Phu Ka.

Chapter 3: Morphology and Gonad Development of Normal Soldiers and Soldier Neotenics of *Zootermopsis nevadensis*

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Hypotheses

Normal soldiers and soldier neotenics will differ significantly in gonad size and in external morphology.

Gonad size will be correlated with measurable external features in both sexes of normal soldiers and soldier neotenics.

Introduction

Soldiers are a non-reproductive defensive caste in termites (though they may sometimes have other roles (Traniello, 1981), and are not always the exclusive defensive caste in a colony (e.g. Nel, 1968, Thorne, 1982, Haverty and Thorne, 1989, Polizzi and Forschler, 1998, Delphia et al., 2003)). They generally have large, rectangular heads with enlarged mandibles (reviewed by Weesner, 1969a). They are found in all termites, except in the Termitid subfamily Apicotermatinae, in which they have been secondarily lost (Inward et al., 2007). Primitive termites of the family

Termopsidae retain developmental plasticity which allows all castes to become reproductive, either through development into an alate (winged dispersal form), or through moults into a non-dispersive neotenic reproductive (Light & Weesner, 1951; Noirot, 1985; Noirot & Pasteels, 1987; Roisin, 2000). Although they possess the prothoracic glands needed for moulting, soldiers are the exception to this flexibility, and are considered a “terminal caste” because they do not moult again (Noirot & Pasteels, 1987). However, in six species of primitive Termopsid termites, (Termopsinae: *Archotermopsis wroughtoni* Desneux, *Zootermopsis angusticollis* (Hagen), *Zootermopsis nevadensis* (Hagen), *Zootermopsis laticeps* (Banks); Stolotermitinae: *Stolotermes brunneicornis* (Hagen), *Stolotermes ruficeps* Brauer), some neotenics have soldier-like morphological characteristics, and are called *reproductive soldiers*, or *soldier neotenics* (reviewed by Myles, 1986). These soldier-like neotenics are phylogenetically rare, and have been reported only occasionally in most of the six species, but they are found fairly commonly in *A. wroughtoni* and *Z. nevadensis* (Imms, 1919; Thorne et al., 2003). Soldier neotenics possess large mandibles similar to those of normal soldiers but have fully developed gonads (Imms, 1919; Heath, 1928; Castle, 1934b; Myles, 1986). Additionally, in *Zootermopsis nevadensis* they exhibit behavior more similar to a neotenic than a soldier (Heath, 1928; Thorne et al., 2003).

Because soldier neotenics occur only in the most socially and developmentally primitive termites, they are considered probable evolutionary relicts of an

intermediate form of soldiers: a stepping-stone on the way to obligatory sterility and altruistic defense (Myles, 1986; Thorne et al., 2003).

Soldier neoténics, while possessing the generalized defensive soldier form, typically have slight differences in external morphology that distinguish them from normal soldiers. These differences include a slightly rounder head shape and shorter, more curved mandibles (Heath, 1928; Myles, 1986). However, there is often individual variation in external morphology (Light, 1943; Morgan, 1959).

In this chapter I examine the relationship between external morphology and internal gonad development in normal and reproductive soldiers in *Zootermopsis nevadensis*, the species in which soldier neoténics have been studied most intensively (Heath, 1928; Light, 1943; Thorne et al., 2003). Using measurements of several external features as well as gonad dimensions, I quantify differences between normal soldiers and soldier neoténics (referred to collectively henceforth as “soldier morphs”). I will also correlate external morphological features with gonad size in soldier morphs.

Methods

I classified individuals from 68 *Zootermopsis nevadensis nevadensis* colonies as “soldier” (144) or “soldier neoténic” (47) prior to dissection, based upon external morphology and observed colony role. An additional 27 individual did not fit clearly into either category. The majority (192) were from outbred laboratory colonies, which in turn had been bred from colonies started by alate pairs that had emerged

from wild colonies near Placerville, CA (El Dorado County). I collected eight individuals directly from these stock colonies. Sixteen were collected from the field in October 2007 from Eldorado National Forest (El Dorado County, CA). I preserved sixteen individuals in ethanol without fixative, and fixed 79 in Bouin's solution (composed of 37% formaldehyde (24% by weight), picric acid (71%), and glacial acetic acid (5%): BBC Biochemical Corporation) for at least an hour before transfer to 80% ethanol, and I fixed and stored 115 in Pampel's solution (composed of glacial acetic acid (7% weight per volume), anhydrol (27%), formaldehyde (4.4-16%), and water (61.6-79%): Bioquip Products). Eight had been preserved in an unknown manner.

I took the following external measurements from each individual, using a eyepiece-mounted micrometer on a Leica dissecting microscope: width of head capsule, length of head capsule, length of left mandible from condyle to apex, length of labrum, width of labrum, wingbud length (if present), width of postmentum at narrowest point, width of postmentum at widest point, length of postmentum, length of eye, and width of eye. I also recorded sex. After completing the external measurements, I pinned each termite to a paraffin-filled Petri dish and made a longitudinal incision on the dorsal side. I injected a solution of Nile Blue dye and water into the body cavity with a syringe and then rinsed the cavity with 70% ethanol after several seconds. I filled the dish with enough 70% ethanol to partially cover the specimen and took the following measurements: females---width of ovary at midpoint, width of ovary at widest point, length of ovary from tip to base of posterior ovariole, number of eggs;

males—widest diameter of testis, narrowest diameter of testis. I calculated ratios of head, labrum, and testes lengths and widths for each individual as a measurement of “roundness.” I also calculated the ratio of the mandible length to the labrum length. These morphometrics were suggested by N.L.Breisch (personal communication) as useful differentiating characteristics for soldier morphs as well as by the published anatomical work on the reproductive system of *Z. nevadensis* and other termites by Child (1934) and Weesner (1969b).

Data Analysis

I analyzed all data using SAS 9.1 for Windows. I chose a p-value of 0.05 as the measure of statistical significance for all analyses.

Contrasting Soldier Morphology with Soldier Neotenic Morphology

I used the GLM procedure to conduct all analyses of variance due to the unbalanced nature of the data set. I applied a multivariate analysis of variance (MANOVA) to determine which variables differed significantly between soldiers and soldier neotenics as well as to determine an overall difference in morphology between these castes. I conducted the MANOVA separately for each sex due to sex-specific missing data. I calculated means for each significant variable by caste, after combining data for both sexes for those variables that had no significant sex effect or caste by sex interactive effect.

Correlating External Morphology with Gonad Size

I conducted correlation analysis on the full data set (including ambiguous individuals) to determine correlations between external morphometry and gonad morphometry. This analysis was meant to look at the continuum of morphology across all soldier morphs. Correlations were univariate, and thus may result in an overall type I error rate greater than the pair-wise rate of 0.05.

I conducted a second correlation analysis separately for soldier neotenics and soldiers (excluding ambiguous individuals) to determine if the two soldier morphs had different allometries.

Presence of Eggs and Wingbuds

I coded number of eggs and wingbud length as binary variables (0=absence, 1=presence). I used Fisher's Exact Test to test the frequency of these binary data for each caste.

Results

Morphological Differences Between Soldiers and Soldier Neotenics

The results of the MANOVA for each individual variable, as well as the means by sex and caste and means pooled by caste, are listed below in Tables 4a and 4b. Pooled means were calculated after grouping male and female data together for those variables with no significant sex difference. For both male and female morphology,

there was a significant overall caste effect (Wilks' Lambda statistic, $p < .0001$).

Although most individual variables were significantly different between castes (Table 4a), there was no caste effect for several morphological variables. For males, these included labrum length ($p = .7438$), wingbud length ($p = .3265$), and the ratio of testis length to width ($p = .9193$). Insignificant variables in females were labrum length ($p = .1484$), eye height ($p = .2373$), eye width ($p = .1506$), wingbud length ($p = .5836$), and the narrowest width of the postmentum ($p = .1168$).

Table 4a. Results of MANOVA and descriptive statistics for each morphological variable measured in *Z. nevadensis*, by sex. Variables with significant sex or caste by sex effects are excluded from the table. Variables with p-values less than .05 were considered significant and are highlighted in bold. There was a significant overall caste effect for both males ($p < .0001$) and females ($p < .0001$) using Wilks' Lambda statistic.

Table 4a: Morphological differences between soldiers and soldier neotenics by sex in *Z. nevadensis*

Variable	P-value of caste effect (males)		P-value of caste effect (females)	
	Mean (cm) ± SE, (n) [male soldiers]	Mean (cm) ± SE, (n) [male soldier neotenics]	Mean (cm) ± SE, (n) [female soldiers]	Mean (cm) ± SE, (n) [female soldier neotenics]
Head Length	<.0001		<.0001	
	.40±.0066 (84)	.31±.0057 (38)	.40±.0061 (58)	.31±.010 (9)
Head Width	.0004		.0088	
	.30±.0040 (84)	.27±.0047 (38)	.30±.0036 (60)	.26±.0090 (9)
Mandible Length	<.0001		<.0001	
	.32±.0047 (82)	.24±.0044 (38)	.32±.0040 (60)	.24±.010 (9)
Labrum Length	.7438		.1484	
	.063±.00089 (82)	.065±.0013 (38)	.065±.00084 (60)	.068±.0022 (9)
Postmentum Length	<.0001		.0009	
	.28±.0055 (83)	.20±.0053 (38)	.28±.0062 (59)	.21±.012 (9)
Postmentum Width (at widest point)	<.0001		.0019	
	.11±.0014 (83)	.099±.0015 (38)	.11±.0012 (60)	.099±.0039 (9)
Postmentum Width (at narrowest point)	.0010		.1168	
	.064±.00066 (83)	.069±.0014 (38)	.065±.00094 (60)	.067±.0024 (9)
Ratio Mandible length: labrum length	<.0001		<.0001	
	5.23±.12 (81)	3.80±.072 (38)	5.05±.10 (60)	3.59±.15 (9)
Testes Diameter (smallest)	<.0001			
Testes Diameter (largest)	<.0001			
Ratio Testes (D1:D2)	.9193			
Ovary Length			<.0001	
Ovary Width (midpoint)			<.0001	
Ovary Width (widest point)			<.0001	

Table 4b: Morphological Differences Between Soldiers and Soldier Neotenics of *Z. nevadensis*, Sexes Pooled

Variable	Mean (cm) \pm SE, (n) [soldiers]	Mean (cm) \pm SE, (n) [soldier neotenic]
Head Length	.40 \pm .0046 (142)	.31 \pm .0050 (47)
Head Width	.30 \pm .0028 (144)	.27 \pm .0041 (47)
Mandible Length	.32 \pm .0032 (142)	.24 \pm .0040 (47)
Labrum Length		
Postmentum Length	.28 \pm .0041 (142)	.20 \pm .0049 (47)
Postmentum Width (at widest point)	.11 \pm .00096 (143)	.099 \pm .0014 (47)
Postmentum Width (at narrowest point)	.064 \pm .00055 (143)	.068 \pm .0012 (47)
Ratio Mandible length: labrum length	5.15 \pm .081 (141)	3.76 \pm .065 (47)
Testes Diameter (smallest)	.033 \pm .0012 (60)	.074 \pm .0036 (38)
Testes Diameter (largest)	.042 \pm .0015 (72)	.094 \pm .0050 (38)
Ovary Length	.15 \pm .0068 (53)	.36 \pm .051 (7)
Ovary Width (midpoint)	.019 \pm .0013 (50)	.070 \pm .01 (7)
Ovary Width (widest point)	.025 \pm .0013 (51)	.087 \pm .012 (7)

Table 4b. Means for significant morphological variables for soldiers and soldier neotenics in *Z. nevadensis*. Males and females were pooled for each caste for variables with no significant sex or sex-by-caste interaction.

There was no overall sex effect ($p=.1467$), however several individual variables were significantly different by sex (Fig. 6). These included width of the eye ($p=.0442$), the ratio of the labrum length to width ($p=.0072$), labrum width ($p=.0180$), and ratio of the head length to width ($p=.0064$).

**Figure 6: Morphological Differences Between Male and Female
Soldier Morphs in *Z. nevadensis***

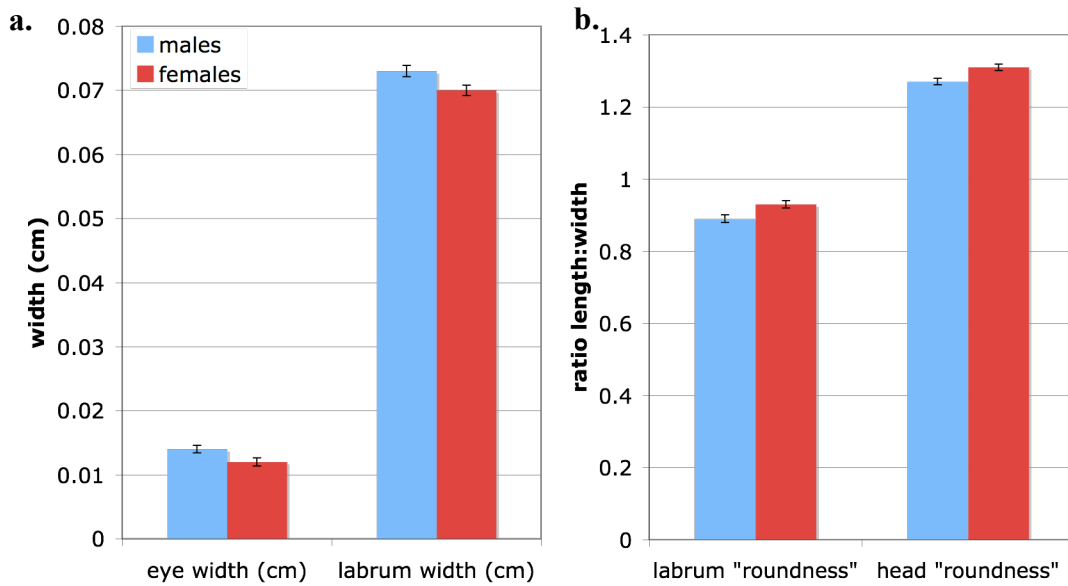


Fig. 6. Significant morphological differences between males and females of *Z. nevadensis* soldier morphs (pooled soldiers and soldier neotenucs). Although there was no overall sex effect ($p=.1467$), **a.** males had wider eyes ($p=.0442$) and labrums ($p=.0180$) than females, **b.** as well as more oblong labrums ($p=.0072$) and rounder heads ($p=.0064$) than females. (A ratio of 1 indicates a perfect circle.) Error bars indicate standard errors.

There was no overall caste by sex interaction ($p=.4868$), although one variable, eye height, had a significant interaction ($p=.0305$, Fig. 7).

Figure 7: Caste by Sex Interaction in Soldier Morph Morphology of *Z. nevadensis*

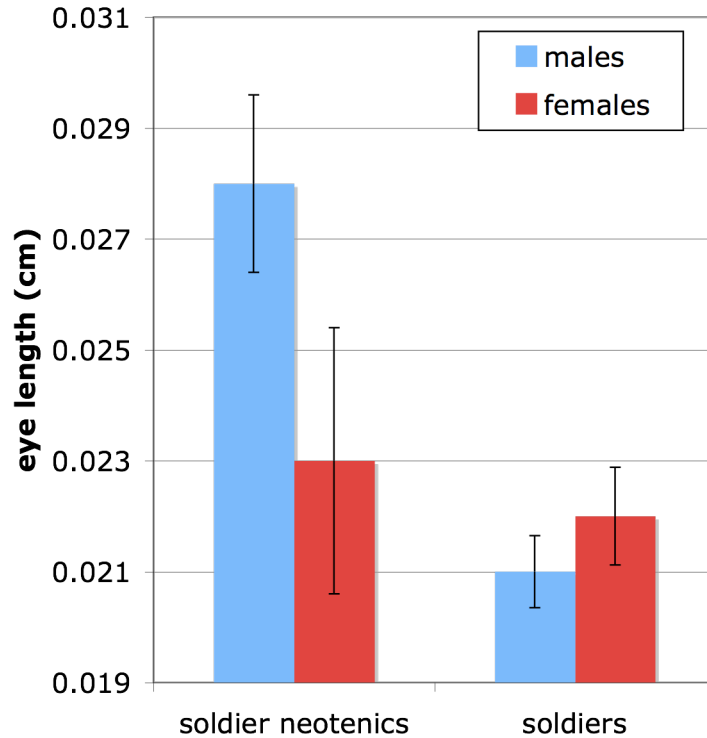


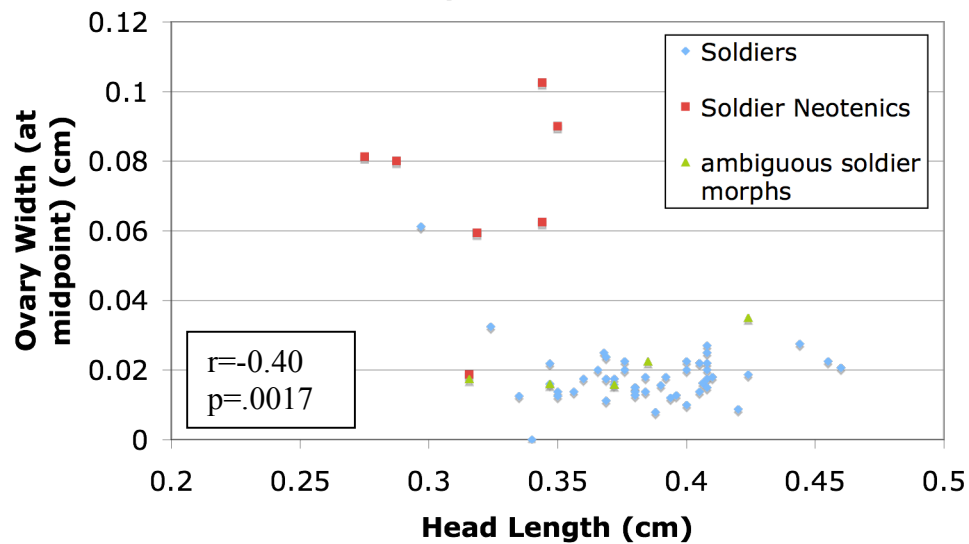
Fig. 7. Significant caste by sex interaction in soldier morphs (normal soldiers and soldier neotenics) of *Z. nevadensis* ($p=.0305$). Although there was no overall caste by sex interaction in the morphological data ($p=.4868$), the two male soldier morphs have a significantly greater difference in eye length than do females. Error bars indicate standard errors.

Four of eight female soldier neotenics had at least one egg: no female normal soldiers had eggs ($p=1.65 \times 10^{-4}$, Fisher's Exact Test). There was no significant difference between possession of wingbuds by caste ($p=.55$, Fisher's Exact Test).

Correlations Between External and Internal Morphology

In female soldier morphs of *Z. nevadensis*, head length (Fig. 8), head “roundness,” left mandible length, labrum length, labrum width, labrum “roundness” (Fig. 9), postmentum width at the narrowest point, eye width, and the ratio of mandible length to labrum length (Fig. 10) were significantly correlated with at least one measure of ovary size.

**Figure 8: Relationship between Ovary Width and Head Length
in Female Soldier Morphs of *Z. nevadensis***



“Roundness” in Female Soldier Morphs of *Z. nevadensis*

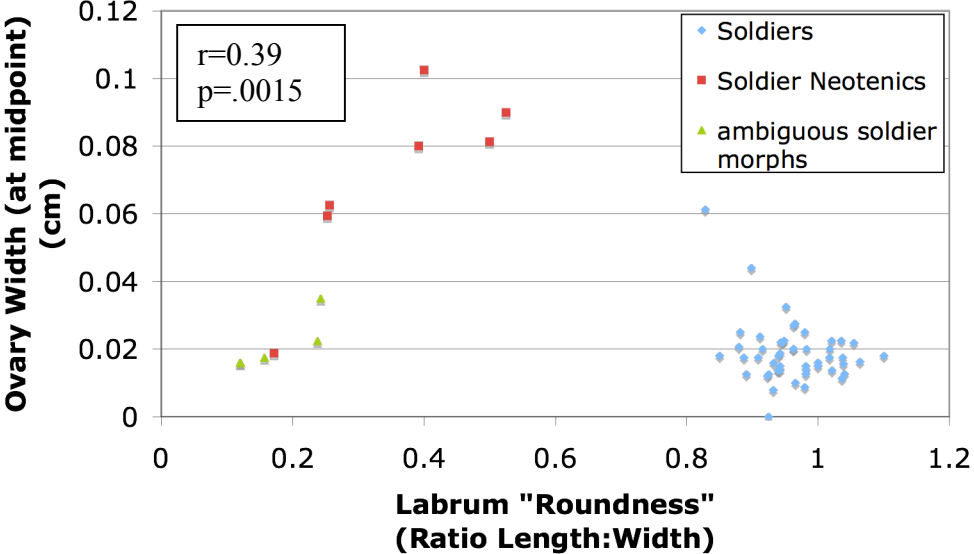


Fig. 9: Correlation between ovary width (at widest point across) and labrum “roundness” (ratio of labrum length to width) in female soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

**Figure 10: Relationship between Ovary Length
and the Ratio of Mandible Length to Labrum Length
in Female Soldier Morphs of *Z. nevadensis***

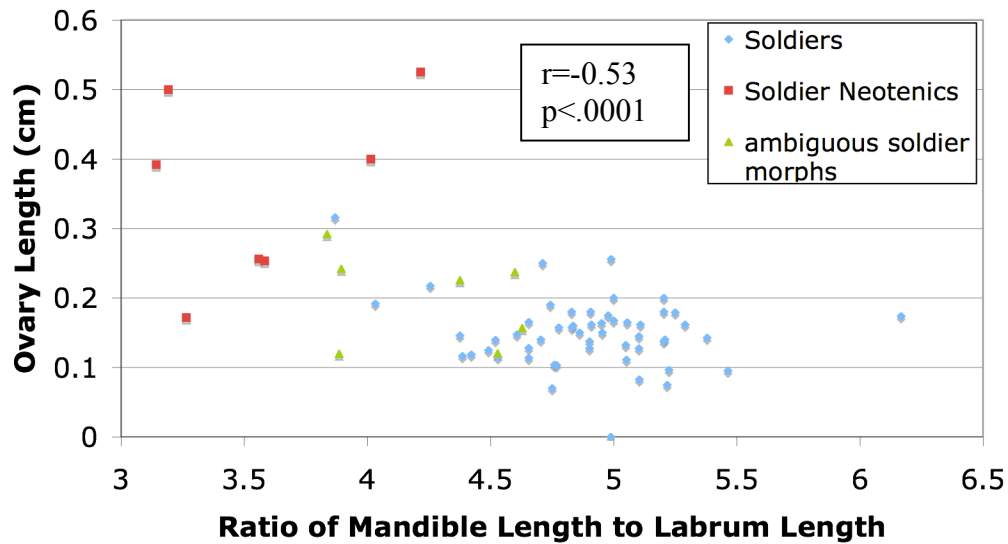


Fig. 10. Correlation between ovary length and the ratio of mandible length to labrum length in female soldier morphs (soldiers and soldier neotenuics) of *Z. nevadensis*.

For male soldier morphs, head length (Fig. 11), head width, head “roundness,” left mandible length, labrum width, labrum “roundness” (Fig. 12), postmentum length, postmentum widths at the widest and narrowest points, eye length, eye width, and the ratio of mandible length to labrum length (Fig. 13) were significantly correlated with at least one measure of testis size or shape. (See Appendix for graphs of all significant correlations not included here.)

**Figure 11: Relationship between Testis Diameter and Head Length
in Male Soldier Morphs of *Z. nevadensis***

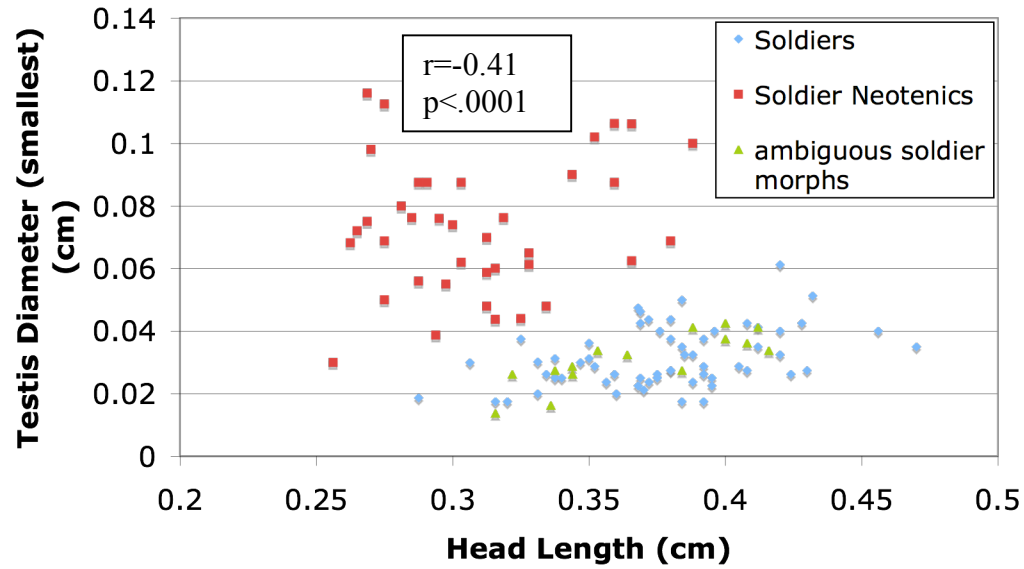


Fig. 11. Correlation between testis diameter (smallest) and head length in male soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

Figure 12: Relationship between Testis Diameter and Labrum

“Roundness” in Male Soldier Morphs of *Z. nevadensis*

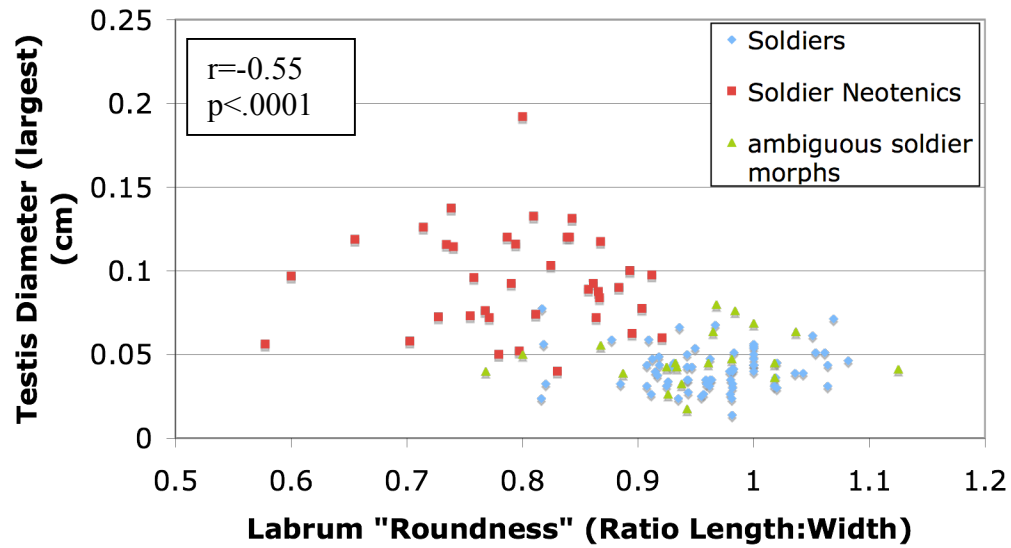


Fig. 12. Correlation between testis diameter (largest) and labrum “roundness” (ratio of labrum length to width) in male soldier morphs (soldiers and soldier neotenic) of *Z. nevadensis*.

**Figure 13: Relationship between Testis Width
and the Ratio of Mandible Length to Labrum Length
in Male Soldier Morphs of *Z. nevadensis***

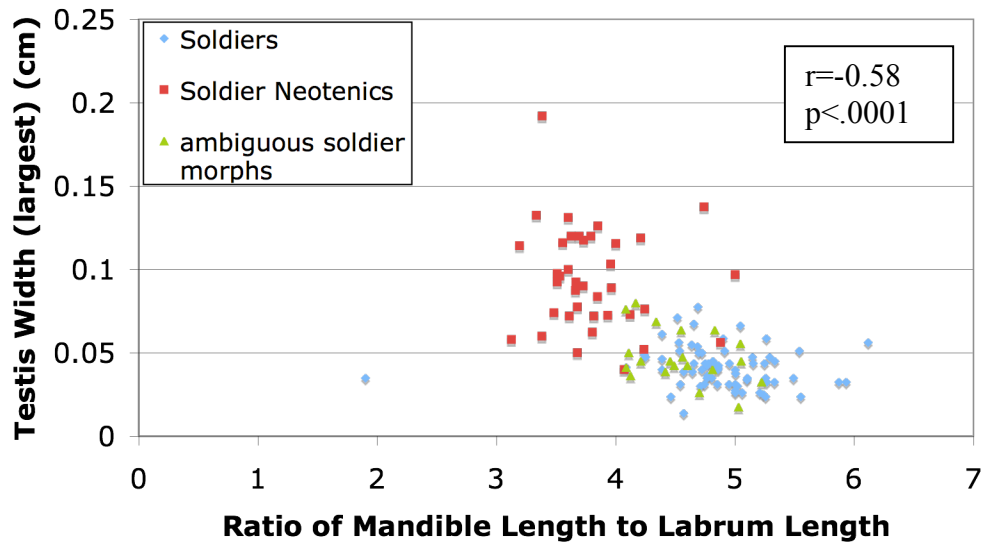


Fig. 13. Correlation between testis width (largest) and the ratio of mandible length to labrum length in male soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

When correlations were analyzed separately for soldiers and for soldier neotenics to look for allometric differences between soldier morphs and soldiers that might be obscured by a combined analysis, there were no variable combinations that were significant for both soldiers and soldier neotenics, yet that had correlation coefficients with opposite signs for each soldier morph. A number of variable combinations were significantly correlated for soldiers but not for soldier neotenics when analyzed separately (Table 5). Only one correlation was significant among soldier neotenics

and not among soldiers (smallest testis diameter with mandible length: soldiers $p=.25652$, $r=.25652$, $n=51$; soldier neoténics $p=.0354$, $r=.78773$, $n=7$).

Table 5. Correlations between gonad size measurements and external morphological measurements of soldier morphs (soldiers and soldier neoténics) of *Z. n. nevadensis*. Gonad size variables are in the first row, while external morphological variables are listed in the first column. For each combination of variables, the Pearson correlation coefficient (r), the p -value, and n is listed. P -values of less than 0.050 were considered significant and are highlighted. A single asterisk denotes correlations that were significant only for soldiers, when analyzed separately for soldiers and soldier neoténics. A double asterisk indicates a correlation that was significant only for soldier neoténics when analyzed separately for soldiers and soldier neoténics.

**Table 5: Correlations Between External Morphology and Gonad Size
in Soldier Morphs of *Z. nevadensis***

External variables	Internal variables					
	Testes diameter (smallest)	Testes diameter (largest)	Ratio testes (D1:D2)	Ovary length	Ovary width (midpoint)	Ovary width (widest point)
Head length	r=-0.41	r=-0.36	r=0.072	r=-0.27	r=-0.40	r=-0.34
	p<.0001	p<.0001*	p=.46	p=.028*	p=.0017	p=.0079
	n=113	n=130	n=106	n=65	n=60	n=61
Head width	-0.22	-0.16	0.078	-0.12	-0.22	-0.15
	p=.017*	p=.075*	p=.42	p=.32*	p=.090	p=.25
	n=113	n=130	n=106	n=67	n=62	n=63
Ratio head (L:W)	-0.45	-0.47	0.0079	-0.34	-0.43	-0.42
	p<.0001**	p<.0001	p=.94	p=.0056	p=.0006	p=.0008
	n=113	n=130	n=106	n=65	n=60	n=61
Mandible length	-0.52	-0.50	-0.017	-0.37	-0.46	-0.39
	p<.0001*	p<.0001*	p=.86	p=0.0020	p=.0002	p=.0017
	n=112	n=128	n=105	n=67	n=62	n=63
Labrum length	0.087	0.12	-0.024	0.33	0.34	0.30
	p=.36*	p=.18*	p=.81	p=.0071*	p=.0073	p=.019*
	n=111	n=128	n=104	n=67	n=111	n=63
Labrum width	0.55	0.57	-0.0029	0.50	0.54	0.47
	p<.0001*	p<.0001*	p=.98	p<.0001*	p<.0001*	p=.0001*
	n=111	n=128	n=104	n=67	n=62	n=63
Ratio labrum (L:W)	-0.56	-0.55	-0.0028	-0.33	-0.39	-0.36
	p<.0001	p<.0001	p=.98	p=.0057*	p=.0015	p=.0037
	n=111	n=128	n=104	n=67	n=62	n=63
Postmentum length	-0.52	-0.49	-0.0064	-0.23	-0.31	-0.24
	p<.0001*	p<.0001*	p=.95	p=.061	p=0.17	p=.06
	n=111	n=128	n=104	n=65	n=60	n=61
Postmentum width (widest point)	-0.34	-0.31	0.063	-0.10	-0.19	-0.098
	p=.0002	p=.0003*	p=.53	p=.41*	p=0.14	p=.45*
	n=111	n=128	n=104	n=66	n=61	n=62
Postmentum width (narrowest point)	0.31	0.34	0.056	0.27	0.25	0.25
	p=.0009*	p<.0001*	p=.57	p=0.026*	p=.055	p=.049*
	n=111	n=128	n=104	n=66	n=61	n=62
Eye length	0.29	0.27	-0.063	0.23	0.24	0.18
	p=.0017*	p=.0021	p=.52	p=.057*	p=.063*	p=.16*
	n=113	n=130	n=106	n=67	n=62	n=63
Eye width	0.41	0.36	-0.074	0.36	0.37	0.33
	p<.0001*	p<.0001	p=.45	p=.0027*	p=.0033*	p=.0083*
	n=113	n=130	n=106	n=67	n=62	n=63
Ratio mandible length to labrum length	-0.57	-0.58	-0.0060	-0.53	-0.60	-0.53
	p<.0001	p<.0001	p=.95	p<.0001*	p<.0001*	p<.0001*
	n=111	n=127	n=104	n=67	n=62	n=63

Discussion

Solder neotenic of both sexes had smaller, rounder heads than soldiers, shorter (or more curved) mandibles, longer and more rectangular postmentums, wider and more oblong labrums, larger eyes, and a lower mandible-to-labrum ratio than soldiers. Soldier neotenic also had larger gonads than soldiers and were more likely to produce eggs than soldiers (none of which were found with eggs). There was no difference between castes in terms of wingbud presence.

Although head size and shape has been known to be associated with reproductive status (Heath, 1928; Myles, 1982), this is the first record of other morphological differences in soldier morphs besides differences in gonad size.

A number of external morphological measurements may be used as indicators of gonad size in both sexes, including head length, head “roundness,” left mandible length, labrum width, labrum “roundness,” postmentum width at the narrowest point, eye width, and the ratio of mandible length to labrum length. For males, head width, postmentum length, postmentum width at widest point, and eye length are also good correlates of testis size. In females only, labrum length also can predict ovary size. The separate analyses of correlations by caste indicate, however, that there may be slightly different allometries between soldier morphs. This is reflected in correlations that were significant for one caste but not the other (usually significant for soldiers and not for soldier neotenic). However, no variable was significant for both castes but with opposite signs on the Pearson coefficient, which indicates that at least there

are no glaring allometric differences between soldier morphs. Because sample size was much smaller for soldier neotenics than for soldiers, the lack of significant correlations for this caste may merely be an artifact of small samples. This is upheld by the fact that many of the same variables I found to be significant predictors of gonad size in the (grouped) correlation analysis were also significantly different between castes in the MANOVA.

Graphs of several of (grouped) correlations indicate that there is not necessarily a clear delineation in morphology between soldiers and soldier neotenics (Figs. 8-13, and Appendix). Overlap between morphology of these soldier morphs demonstrates that while some individuals may be easily classified as either reproductive or sterile, determining gonad development of others remains difficult based only on external morphology. The 27 individuals I could not classify clearly as soldiers or soldier neotenics were for some morphometrics intermediate in morphology between soldiers and soldier neotenics (e.g. Fig. 10, Fig. 13). Often, though, many of these ambiguous individuals grouped with sterile soldiers, and individuals that had been classified as normal soldiers proved to have somewhat developed gonads upon dissection (e.g. Fig. 8-10). There were also several examples of individuals that had been classified as soldier neotenics that had minimal gonad development (e.g. Fig. 8-13). These results suggest that soldier morphs in primitive Termopsids represent a continuum of morphology and gonad development between the more common “sterile” soldiers, and relatively rare reproductive soldier neotenics. Morgan (1959) documented variation in head shape in the “emergency soldiers” of *Stolotermes ruficeps* Brauer,

though he did not mention gonad development or reproductive status in these individuals. Similarly, Light (1943) noted a range of “intergrades” in *Z. nevadensis*, which he regarded as intercastes between soldiers, juveniles, nymphs, and neotenics. My results suggest that some individuals’ potential reproductive ability can be revealed only by dissection or close observation of behavior. It should be noted, however, that I found eggs only in females classified as soldier neotenics. (Because I did not look for sperm in males, I cannot say how many males were actively reproducing.)

The idea of soldier neotenic as an intercaste implied by Light’s work (1943) is also suggested by recent work with *Z. nevadensis*, in which JHA was applied to nymphs and induced the development of nymph/soldier intercastes (Miura et al., 2003). These individuals look similar to natural soldier neotenics of the species, due to a small, round head, developed gonads, and short, curved mandibles. The intercastes in Miura et al.’s work had a range of morphologies, depending on the nymphal stage at which the JHA was applied. However, they possessed wings or wingbuds. Wings or wing bracts have never been recorded in a naturally occurring soldier neotenic; wingbuds are rare, and not more common in soldier neotenics than in soldiers.

It is likely that modern soldier neotenics represent the first step in soldier evolution, and that the loss of fertility in soldiers was secondary to the development of large mandibles and heavily sclerotized heads, needed by primitive termites for use in intercolony interactions (Thorne et al., 2003), though Roisin (1999) has suggested

that soldier neoteny's distinctive morphology may merely be a non-adaptive accident of the dual roles of JH as both a stimulus for soldier development as well as a gonadotropic hormone in reproductives. The secondary loss of reproductive capacity after the evolution of soldier morphology also appears to have occurred in aphid (Stern & Foster, 1997), thrips (Chapman et al., 2002) and ant soldiers (Urbani, et al., 1996).

Further study is needed to elucidate the developmental pathway of soldier neoteny and to determine if they result from a combination of developmental and/or social signals, or if they develop in response to a single trigger. Because soldier neoteny is considered relictual transitional forms reflecting the evolutionary history of soldiers, this work implies that soldier development may have been much more flexible in the past than in most extant termites.

Chapter 4: Development of Soldier Neotenic in *Z. nevadensis*

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Introduction

Zootermopsis nevadensis (Hagen) (Isoptera: Termopsidae) is a basal termite (Inward et al., 2007) that retains a primitive life-history (Abe, 1987) and developmental and behavioral flexibility (Imms, 1919; Noirot, 1985; Noirot & Pasteels, 1987; Rosengaus & Traniello, 1993; Thorne & Traniello, 2003). These relictual characteristics make the species one of the best living models to investigate the evolution of eusociality, because termites lack extant subsocial taxa (Thorne, 1997; Thorne & Traniello, 2003). *Z. nevadensis*' flexibility derives from its lack of a true, endpoint worker caste; juvenile "helpers" fill the worker role (Noirot, 1985; Noirot & Pasteels, 1987).

Helpers and nymphs (individuals with developing wingbuds), have the capacity to moult into reproductives with certain colony cues (Light & Weesner, 1951; Noirot, 1985; Noirot & Pasteels, 1987). Usually, these reproductives, called neotenic, retain the general helper or nymphal form as a neotenic ("normal neotenic"). However, in *Z. nevadensis* and five other species of primitive Termopsid termites, (Termopsinae: *Archotermopsis wroughtoni* Desneux, *Zootermopsis angusticollis* (Hagen), *Zootermopsis laticeps* (Banks); Stolotermitinae: *Stolotermes brunneicornis* (Hagen), *Stolotermes ruficeps* Brauer), some neotenic have soldier-like morphological characteristics, and are called *reproductive soldiers*, or *soldier neotenic* (reviewed by Myles, 1986). These soldier-like neotenic are phylogenetically rare, and have been

reported only occasionally in most of the six species, but are found fairly commonly in *A. wroughtoni* and *Z. nevadensis* (Imms, 1919; Thorne et al., 2003), though soldiers morphs of *A. wroughtoni* have never been observed actively reproducing. Soldier neotenics possess large mandibles similar to those of normal termite soldiers but have fully developed gonads (Imms, 1919; Heath, 1928; Castle, 1934b; Myles, 1986) and often behave more similarly to a neotenic than a soldier (Heath, 1928; Thorne et al., 2003). Because soldier neotenics occur only in the most socially and developmentally primitive of termites, they are considered evolutionary relicts of an intermediate form of soldiers: a stepping-stone on the way to obligatory sterility and altruistic defense (Myles, 1986; Thorne et al., 2003).

Normal neotenic development is fairly well understood, but the mechanism of soldier neotenic development is almost completely unknown. In *Z. nevadensis*, normal neotenics usually develop from a small percentage of individuals in the 7th-10th instars (Light & Weesner, 1951), though any individual in a *Zootermopsis* colony with the ability to moult has the capacity to become reproductive (e.g. Castle, 1934a; Light & Weesner, 1951). This moult occurs through a process mediated by juvenile hormone (JH) control of the genome (Greenberg & Tobe, 1985; Elliott & Stay, 2007; Cornette et al., 2008; Elliott & Stay, 2008). Heath (1928) and Castle (1934b) proposed that soldier neotenics derive from individuals developing toward a soldier morphology but that are diverted onto a reproductive pathway before the final molt into a soldier. Roisin has suggested that soldier neotenics' morphology may be a side effect of JH's dual role as both a trigger for soldier development and a gonadotrophic hormone for

reproductives (Roisin, 1999). Application of juvenile hormone analog (JHA) on nymphs of *Z. nevadensis* induces an alate/soldier intercaste which displays gonad development and a morphology very similar to that of naturally-occurring soldier neotenics (Miura et al., 2003). Research in Rhinotermitid termites has shown that JH is higher in workers in colonies without soldiers than in colonies with soldiers (Mao et al., 2005), and that high JH titers in workers can induce neotenic production (Elliot & Stay, 2008). These experiments suggest that the presence of normal soldiers may influence development of normal neotenics and soldier neotenics in Termopsid colonies. However, no data exist to demonstrate if soldier neotenics naturally develop from nymphs (as in the laboratory exogenous JHA applications by Miura et al., 2003), if they diverge from the developmental pathway of normal soldiers, or if they differentiate along a unique developmental pathway.

Although little is understood about the developmental programs of soldier neotenics, it is known that soldier neotenics frequently arise in colonies after intercolony interactions (Thorne et al., 2003). Battles between colonies often occur between incipient Termopsid colonies; many pairs of alates may colonize the same piece of wood, and as these colonies grow, meetings between neighboring colonies are inevitable (Thorne et al., 2002). Previous research has demonstrated that soldier neotenics are effective at assassinating opposing primary reproductives (of the same sex) in intercolony conflicts (Thorne et al., 2003). Because of this behavior, it is possible that soldier neotenics would be more likely than a normal neotenic to

develop as a replacement for a dead king or queen in smaller, younger colonies that would be undergoing frequent interactions in the field.

In this chapter I describe two experiments with laboratory-reared *Zootermopsis nevadensis nevadensis* in which I studied the effects of colony demographics on the differentiation of normal neotenics and soldier neotenics. The first experiment focuses on the effect of colony population size and colony age on the development of neotenic reproductives after the king is removed, and the second explores the developmental pathway through which soldier neotenics develop in a colony to replace a dead king. The king, rather than the queen, was chosen for removal because previous work with *Z. nevadensis* has shown that male soldier neotenics are more likely to appear in a colony than female soldier neotenics (see chapter 3).

The more primitive termites maintain a reproductively flexible state that must have been present as an intermediate form between non-social pre-termites and the rigidly eusocial higher termites that exist today (e.g. Noirot, 1985; Noirot & Pasteels, 1987; Abe, 1987; Thorne, 1997; Thorne & Traniello, 2003). Understanding the development of Termopsids' neotenic forms—especially the relictual soldier neotenic—may provide insights into the evolution of castes and eusociality in the Isoptera.

Part I. Does Colony Size Influence the Production of Normal Neotenic Reproductives
versus Soldier Neotenic Following King Loss?

Hypothesis

Smaller, younger colonies that lose their king will produce relatively more soldier neotenic than larger, older colonies, which will produce more normal neotenic.

Methods

I grouped existing king- and queen-right colonies (lab-reared from alates) into three size and age classes, with 18 colonies of each class. The large/old class consisted of colonies initiated with king and queen pairs either on the 6th of March or the 15th of April 2003, and that varied in population size from 81 to 351 individuals (mean=194, standard deviation=57.9) at their most recent census within approximately one month before beginning the experiment. They were five-and-a-half years old when I began the experiment. The medium class consisted of colonies begun on a range of dates within a nine-month period from July 2005 to May 2006, with the majority of the colonies initiated in March-May 2006. These colonies ranged in size from 46 to 120 individuals per colony (mean=73, standard deviation=26.2) at their most recent census within a week of the beginning of the experiment. They were two-and-a-half to three years old at the start of the experiment. The small/young class consisted of colonies started in May or June of 2008 that ranged in population size from 4 to 23 individuals per colony (mean=12, standard deviation=6.0) and were approximately six months old when I began the experiment.

Because of limitations in available colonies, it was impossible to get an even mix of ancestral lines among the colonies. All of the colonies in the large/old class shared one parent from the same original colony, and the other parent from the same cross of two other specific colonies. All of the colonies in the small/young age class were founded by one primary from a colony with the pedigree of the large/old class, and the other primary bred from unrelated colonies. This means that one of the primary reproductives in each small/young colony potentially originated from one of the colonies in the large/old age class. I used a wide variety of colony ancestries in the medium class. The stock colonies from which each experimental colony in this experiment was bred were started in 2002 and 2003 from alate pairs that had emerged from wild colonies near Placerville, CA (El Dorado County).

I assigned colonies to either a king removal or control treatment, with population sizes evenly distributed between the two groups. There were equal numbers of colonies from each class in both groups. I removed both the king and queen from colonies in both treatments so that I could determine which was the male. In the king removal treatment, I killed and preserved the king in 100% ethyl alcohol. I placed the queen from the king removal group and both primary reproductives from the control group back into their respective colonies. (I removed primaries from the control colonies to control for possible colony disturbance effects.)

I grouped colonies into three blocks of 18 colonies each, with 6 replicates of each class per block. To distribute the colonies in each class, I first sorted them by

population size, and then allocated in sequence one colony per block, repeating this process until all colonies in each class were distributed to a block. I used this methodology to ensure an approximately equal distribution of colony sizes to each block. There were also equal numbers of king removal and control group colonies from each class per block. I then assigned each block to a different technician, so that any inter-person variation in census technique or termite handling would be eliminated from the total variation between colonies.

I randomly arranged colonies in stacks on a lab shelf, each stack three or four colonies high. I placed small/young and medium class colonies in larger, lidded containers to make them equal in container size to the large/old colonies to facilitate stacking, as well as to keep the colonies moist. I randomly shuffled colonies at least once per month to eliminate possible effects of height, neighbor, or microclimate.

I fed colonies with chunks of decayed white birch (*Betula papyrifera*) that was soaked in distilled water for 1-2 hours, and sprayed colonies with distilled water as needed.

I censused colonies approximately once every four weeks to document reproductive development, egg presence and total population size. To census, I placed each individual onto a Petri dish covered in a wetted paper towel. I broke open large chunks of wood to remove the termites inside for the census. I gave each new reproductive a uniquely-colored dot of Testor's paint on its thorax or abdomen to

track persistence of individual reproductives. At each census, I repainted older reproductives needing a paint touch-up in their original color.

I used an exact logistic regression with the SAS procedure LOGISTIC to elucidate the effects of time, age/size class, and population size on the numbers of normal neotenics and soldier neotenics present in the colonies at the six-month census, after recoding the response as binary. An exact logistic regression was necessary because of small sample sizes that prevented maximum likelihood estimates from converging.

I also analyzed the survival of each individual reproductive using a logistic regression in the SAS procedure GLIMMIX, which I used because it allows for random factors in the analysis (blocks, in this case). Sample size was large enough to allow convergence with non-exact procedures such as GLIMMIX. For the analysis, I coded each new reproductive that appeared at the 5-month census or before as either surviving or not surviving through the 6-month census.

I removed from both analyses all colonies in which either primary reproductive died during the experiment.

Results

Presence of Male Soldier Neotenics or Male Normal Neotenics at Six-Month Census

Queens died in five king-removal colonies and the king died in one control colony. I do not include the results from these colonies in my results or conclusions.

Five colonies had male soldier neotenics at the 6-month census, while 9 colonies had male normal neotenics (Fig. 14). At this final census, no male soldier neotenics appeared in numbers greater than 1; two colonies had 2 male normal neotenics, however, and 2 colonies had 3 (Fig 15).

Figure 14: Colonies of *Z. nevadensis* with New Male Reproductives

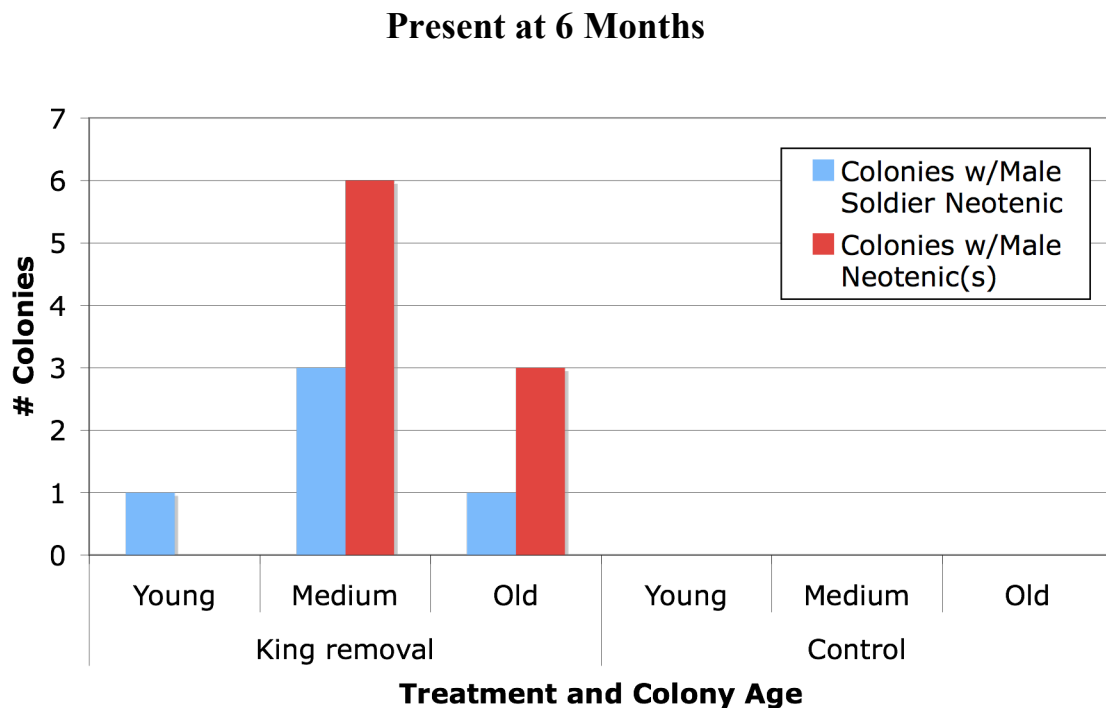


Fig. 14. Number of colonies in king removal treatment or control and in each age class that had a male soldier neotenic or a male normal neotenic after 6 six months.

Neither explanatory variable had a significant effect on male reproductive appearance, except for king removal treatment, which significantly affected male normal neotenic presence ($p=.0006$, $OR=18.554$).

**Figure 15: Reproductive Presence at Six Months
and Colony Population Size in *Z. nevadensis***

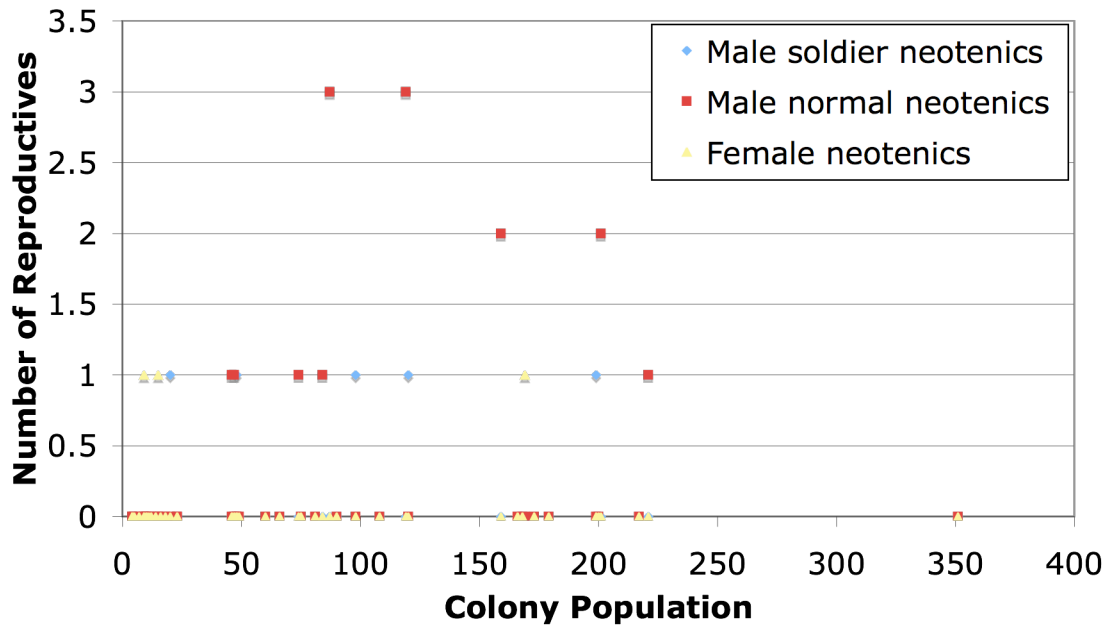


Fig. 15. Colony population at the start of the experiment (month 0) and the number of new male and female reproduces per colony at the six-month census. The data shown here are actual numbers of reproduces; the data were analyzed as a binary response (presence/absence). There was no significant influence of colony population on presence of male soldier neotenics ($p=.73$) or of male normal neotenics ($p=.39$) at the six-month census. Female neotenics were not analyzed.

There was no significant effect of king removal ($p=0.10$), age class ($p=1.0$) (Fig. 14) or starting population ($p=.73$, Fig. 15) on the likelihood of a colony possessing a male soldier neotenic at the 6-month census.

For male normal neotenics, there was no significant effect of age class ($p=.53$, Fig. 14) or starting population ($p=.39$, Fig. 15) on their presence in a colony after 6 months. There was a significant effect of king removal on the presence of male normal neotenics, however ($p=.0006$, $OR=18.554$, Fig. 14).

Survivorship of New Reproductives

There was no significant effect of king removal ($p=.2857$), age class ($p=.4309$), starting population ($p=.7241$), or type of reproductive ($p=.1606$) on the survivorship of new male reproductives to the 6-month census.

Discussion

There was no support for my hypothesis that smaller, younger colonies would produce more male soldier neotenics than male normal neotenics upon king death. This implies that despite soldier neotenics' "assassin" behavior towards neighboring colonies' primary reproductives during intercolony conflict (Thorne et al., 2003), smaller and younger colonies in which these interactions would more likely occur may not have the ability to regulate the type of male replacement reproductive appearing in their colonies. The analysis of male reproductive survivorship data also showed that there was no influence of colony age or size on survival to the 6th month, and neither king removal nor type of reproductive had any effect on survivorship. This suggests that despite male soldier neotenics' more sclerotized head and large mandibles, they are still as vulnerable as male normal neotenics who are not so equipped. However, because I included no interactive effects in the survivorship

analysis due to a lack of model convergence upon inclusion, there could have been an interaction of age or population by reproductive type, which would have been consistent with my hypothesis. Larger sample sizes will be needed to test any possible interactive effects.

It is possible that the small sample size in this experiment could have prevented trends from becoming statistically significant. Of the 21 king-removal colonies that were included in the final analysis, only 14 of them (66.7%) developed apparent male replacement reproductives by the 6th month. More months of censusing might allow more colonies to produce male reproductives. Of the seven king-removal colonies without a male reproductive at six months, however, five of them had eggs, implying that either the queen was storing sperm in her spermatheca, or that the male reproductive in the colony was cryptic and did not have the external morphology characteristic of a normal neotenic or a soldier neotenic.

The one significant effect found in this experiment was the influence of king removal on the odds of a colony producing a male normal neotenic ($p=.0006$, $OR= 18.554$), a long-understood phenomenon (e.g. Grassi & Sandias, 1896; Castle, 1934a; Light, 1943; Light & Weesner, 1951).

In conclusion, male soldier neotenics do not develop more often than male normal neotenics with various colony size and age characteristics, and neither type of reproductive tends to survive longer in a colony. More research with a greater sample

size of colonies and reproductives will be needed to confirm if this is a consistent relationship. If confirmed, however, this would imply that a colony does not have any influence over the type of male reproductive that differentiates to replace a dead king, despite the apparent benefit conferred by the aggressive behavior of soldier neotenics in intercolony interactions (Thorne et al., 2003).

Part II. Does Soldier Neotenic Development Occur as a Divergence From the Developmental Pathway of Normal Soldiers?

Hypothesis

Removing a colony's normal soldiers before removing the king will result in relatively more, and faster, production of soldier neotenics than in control colonies in which only the king is removed.

Methods

I used thirty existing king- and queen-right colonies in this experiment. These colonies had been initiated in the period from July 2005 to October 2007, with the majority of the colonies originating from March through May of 2006. The colonies were 10 months to three years old at the beginning of the experiment. All colonies had exactly two soldiers when the experiment began, and their most recent census (within the previous month) indicated population sizes of 27 to 94 individuals per colony (mean=45, standard deviation=15.5).

The colonies I used in this experiment were bred using a variety of combinations of opposite-sex alate pairs originating from stock colonies that were themselves started in 2002 and 2003 by alate pairs that had emerged from wild colonies near Placerville, CA (El Dorado County).

I split the 30 colonies into two blocks according to starting population size, in order to remove variation based on number of individuals in the colony. I then assigned them to either the king removal treatment or soldier+king treatment with an aim to have an equal number of colonies with similar population sizes in each treatment. I stored the colonies from each block together in one of two larger plastic containers that maintained high humidity.

I removed both soldiers from each colony. I killed and stored the soldiers from the soldier+king removal colonies in 100% ethyl alcohol; whereas I returned the soldiers from the king removal treatment to their colonies within several minutes. One week later, I removed the kings from all colonies into 100% EtOH. I chose this period of time because preliminary experiments showed that one week was about half the minimum time needed for *Z. nevadensis* colonies of this size to develop a new soldier (unpublished data).

I censused the colonies approximately once every four weeks to document reproductive development, soldier development, egg presence and total population size, using the methods described in Part I. One technician was assigned to each

block in order to control for variation in census technique or termite handling between individuals.

Colonies were fed and watered as in Part I.

I analyzed population census data at the sixth month by recoding male soldier neotenic and male normal neotenic numbers as a binary presence/absence per colony. I used the SAS procedure GLIMMIX to perform a logistic regression. I used GLIMMIX for this analysis because of its ability to account for random factors (blocks in this experiment). Sample size was large enough to allow convergence with non-exact procedures such as GLIMMIX.

Results

Male Replacement Reproductives

At the six-month census, six control colonies and one treatment colony had a male soldier neotenic (Table 6). Nine controls and nine treatment colonies had a male normal neotenic (one of these treatment colonies had two). Of these, two control colonies had one of each type of male reproductive. Two control colonies and five treatment colonies had no apparent male reproductives at six months after king removal. Despite a trend towards more soldier neotenic in king removal colonies, there was no significant difference between treatments in the presence of male soldier neotenic ($p=.3454$) or male normal neotenic ($p=.8565$) in a colony after six months.

Table 6: Number of Colonies of *Z. nevadensis* with Different Types of Male Reproductives, by Month and Treatment

Month	Treatment	Number of Colonies		
		Soldier neotenic	Normal neotenic	Both
1	King removal	0	0	0
	Soldier+King removal	0	0	0
2	King removal	1	2	1
	Soldier+King removal	0	2	0
3	King removal	1	3	2
	Soldier+King removal	0	3	0
4	King removal	3	4	1
	Soldier+King removal	1	6	0
5	King removal	4	7	1
	Soldier+King removal	0	10*	0
6	King removal	4	7	2
	Soldier+King removal	1	9*	0

Table 6. The number of colonies possessing only male soldier neotenics, only male normal neotenics, or both types of male neotenics, for each month of censusing and for each treatment. Each asterisk denotes that one colony in that category has two male normal neotenics (no soldier neotenics were found in multiples per colony).

More male reproductives appeared in the colonies throughout time (Table 6). Male replacement reproductives began to appear in colonies at the second month's census. (King removal was considered month zero.) There were only four colonies in which there existed two male reproductives at the same time; of these, one colony had a male soldier neotenic and a male normal neotenic only in months 2 and 3, one colony had both a male soldier neotenic and a male normal neotenic from months 3-6, one colony had a male soldier neotenic and a male normal neotenic in month 6 only, and another colony had two male normal neotenics in months 5 and 6. The data do not

provide any insight into persistence of individual males, because no termites were painted or otherwise tracked as individuals. No colony ever had more than two male neotenics differentiating within the same census period.

Neotenic Head Capsule Scarring

Scars were seen on the head capsules of 11 male neotenics and 1 female neotenic (57.9% and 12.5%, respectively). The difference in frequency of scarring was significant between the two sexes with a Chi-square analysis ($p=0.02$, d.f.=1). Scars appeared as dark lines on the dorsal side of the head capsule. There were often multiple scar lines on one individual. Infliction of these wounds was never observed.

Female Supplementary Reproductives

Female neotenics appeared in three treatment colonies at censuses 3, 4, and 5, although the queen in those colonies was still present. By the final census, the colonies had 5, 2, and 1 female neotenic each. All were normal neotenics rather than soldier neotenics.

Soldier Development

Forty percent (6/15) of king removal colonies (in which soldiers were not killed at month 0) developed more soldiers by the end of the censusing period. Thirty-three percent (5/15) of king removal colonies neither gained nor lost soldiers after six months, and 27% (4/15) lost soldiers (Fig. 16). Of the soldier+king removal colonies (those in which both soldiers were killed at the outset), almost half (46%, 7/15) regained two soldiers. Twenty-seven percent (4/15) only regained one soldier after

six months, and two colonies each (13%, 13%) failed to regenerate any soldiers or had three soldiers by the end of the censusing period (Fig. 16).

Figure 16: Number of Soldiers in Colonies of *Z. nevadensis*

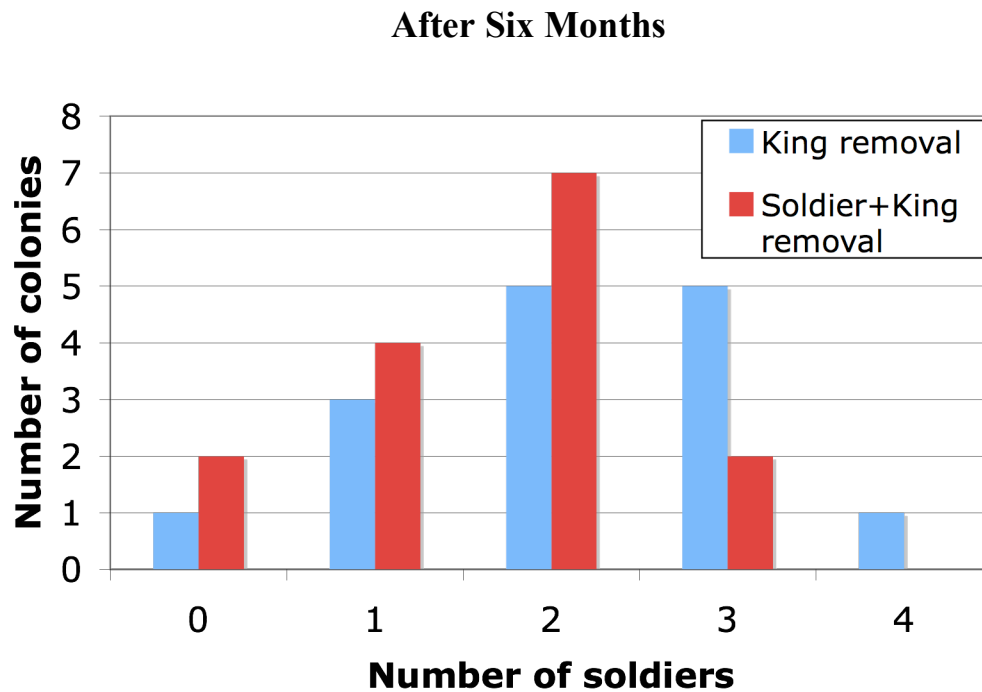


Fig. 16. Number of colonies with varying number of soldiers per colony in both king removal and soldier+king removal treatments at six-month census.

Queen Survival

All queens survived through six months.

Discussion

Presence or absence of soldiers did not influence the likelihood of a colony developing a male soldier neotenic or male normal neotenic. This suggests that male

soldier neotenics are not in fact developmental offshoots of the soldier line, and that soldiers do not affect a colony's ability to produce a male replacement reproductive. These results provide no support to Roisin's hypothesis that soldier neotenic morphology is a developmental accident of "interference" between the two roles of JH: to stimulate soldier development, as well as to stimulate gonad development in reproductives (Roisin, 1999); I would have seen soldier neotenics more often in colonies in which both soldiers and neotenics were actively developing if this were the case.

Because all but three colonies that differentiated male secondary reproductives had only one after six months, this could mean that neotenic males are usually capable of suppressing reproductive development in other males. Census data from this experiment are inconclusive regarding whether the numbers of male neotenics are regulated at the beginning of their development, or whether multiple neotenics are produced and "extras" are killed. However, scars on the head capsules of many neotenics suggest the latter scenario. Because I made no observation of the infliction of these wounds, it is impossible to say who was responsible for the scars. These types of scars are common in *Z. nevadensis* neotenics; although it is unknown which caste is generally responsible for these wounds, the location of the scars in the middle of the dorsal side of the head is consistent with the large jaws of a soldier, and a soldier has been observed inflicting a wound of this type (N.L. Breisch, personal communication). However, not all head scarring seen in this experiment occurred in colonies with soldiers present, so soldiers cannot be the exclusive culprits. In artificial

colonies of *R. speratus*, targeted attacks by workers against nymphoid reproductives were observed, as well as a drop in neotenic numbers after an initial spike, suggesting violent elimination of supernumerary reproductive individuals (Miyata et al., 2004). Although I did not record multiple male neotenic in most colony censuses, I observed that many colonies had multiple individuals with enlarged abdomens before the appearance of a clear reproductive male. This suggests that several individuals might have been developing along a reproductive pathway and most were eliminated.

Because the occurrence of scarring was significantly lower among female than male neotenic, and multiple females coexisted in the colonies in which female neotenic appeared, these data are consistent with reports of the higher tolerance within colonies for multiple females than for multiple males both in *Zootermopsis* as well as in other termites (Pawson & Gold, 1996; Miyata et al., 2004; Brent et al., 2008). In their work in *Z. angusticollis*, however, Brent et al. (2008) found no evidence of violence against female neotenic, such as the scarring seen on one of the females in my work. Female neotenic coexisting with a queen in *Zootermopsis* spp. has been reported; Light and Weesner (1951) observed this situation in 14.9% of *Z. angusticollis* colonies in queen-headed colonies, though Thorne et al. (2003) reported this occurrence only once in seven years in *Z. nevadensis*.

One notable result is the fact that seven of the thirty-two colonies had no apparent male reproductive by the end of the six-month census period. Although most of those colonies had eggs for several months in the beginning of the experiment, all but one

had no eggs in the colony by the last months of the experiment, suggesting that the queen had sequestered the dead king's sperm in her spermatheca and ran out of sperm for egg fertilization after several months. It is surprising however, that no males were able to develop into reproductives during this time in these colonies. *Z. angusticollis* has been shown to need only a week of separation from a king or queen to start producing secondary reproductives, as long as the colony contains individuals who are mature enough to undergo this transition (Light & Weesner, 1951). In the one colony in my experiment without an apparent male neotenic but with eggs at six months, there could have been a cryptic reproductive—that is to say, an individual that appeared to be a normal helper or a normal soldier could have been fertilizing the queen without being detected by the census technician because he did not exhibit the normal neotenic morphology. I noted several slightly fatter individuals in this colony in the last three months of censusing, so it is possible that some or all of these individuals were the ones responsible.

Conclusions From Sections I and II on the Development of Soldier Neotenic in Z. nevadensis

In conclusion, the developmental pathway of the relictual soldier neotenic of *Z. nevadensis* is still obscure; neither colony size, colony age, nor the presence of normal soldiers had any significant effect on the likelihood of this replacement reproductive caste to differentiate upon death of a king instead of a normal neotenic. Because of limited sample sizes in both experiments, more research with a greater number of colonies and reproductives will be needed to confirm if these patterns are

consistent. It is possible that there is not one specific developmental pathway for soldier neotenic, making its detection difficult; similarly, there may be a multitude of colony signals with complex interactions that affect soldier neotenic's development. Microarray analysis of active genes in soldier neotenic would be a useful step to determine if the same genes responsible for the growth of the large mandibles and heavily sclerotized heads of soldier neotenic are the same that are upregulated in the development of normal soldiers (Scharf et al., 2003; Hojo et al., 2005). It is possible that entirely separate genetic programs are responsible for the soldier-like morphology of normal and reproductive soldiers, which would explain why no more soldier neotenic developed in colonies that had lost soldiers than those that had not. Further studies are also necessary to better elucidate the role of soldier neotenic in colonies of different ages. I assumed that they would be more beneficial in smaller, younger colonies, which would more frequently undergo intercolony interactions in which soldier neotenic have proven to be effective "assassins" (Thorne et al., 2003). However, there may be different benefits to the differentiation of a normal neotenic in a young colony that would obscure this signal, or, alternatively, colonies or individuals simply may not be able to influence this developmental "choice." More research will continue to shed light on the development of this relic caste that may hold a key for understanding the evolution of sterile soldiers during the evolution of eusociality in termites (Myles, 1986; Thorne, 2003).

Chapter 5: Soldier Neotenics' Role in Interactions with Colonies Containing Normal Neotenic Reproductives in *Z. nevadensis*

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Hypothesis

Soldier neotenics are more likely to survive intercolony interactions than are normal neotenics.

Introduction

Termites (order Isoptera) are xylophagous insects, monophyletic with roaches (Inward et al., 2007; Legendre et al., 2008), and are ancestrally eusocial. In most termite colonies, reproduction is monopolized by the primary reproductives (king and queen). In many termite species, these “primaries” have very long lifespans (reviewed in Thorne et al., 2002), which would seem to limit opportunities for their helping offspring in the nest to develop into reproductives upon the parents' death. However, basal termite lineages with primitive life-histories face frequent intraspecific conflict, in which primary reproductives' lifespans are drastically

shortened (Thorne et al., 2002; Thorne et al., 2003). The prospect of inheriting an established nest and its resources has been proposed as a potential driving force for the evolution of eusociality (Wilson, 1971; Alexander et al., 1991; Shellman-Reeve, 1997; Thorne 1997; Thorne & Traniello, 2003; Korb, 2008)

Dampwood termites, because of the retention of ancestral life-histories, colony dynamics and flexible development, provide the best living model species for understanding conditions that may have led to the evolution of eusociality in termites (Thorne 1997; Thorne & Traniello, 2003). Termites of the primitive family Termopsidae are one-piece nesters, which means that a colony lives and eats exclusively within one piece of wood (Abe, 1987). Battles between colonies often occur between incipient Termopsid colonies; many pairs of alates may colonize the wood resource, and as these colonies grow, meetings between neighboring colonies are inevitable (Thorne et al., 2002). The Accelerated Inheritance Hypothesis for the evolution of eusociality in termites states that the common occurrence of interactions between growing termite families sharing the same piece of wood—resulting in early death of primary reproductives—provides a means by which young helper offspring might gain a chance to develop and fill the reproductive vacancies as replacement reproductives (Thorne et al., 2003).

Most replacement reproductives in termite colonies are neotenics, which develop from immature forms through at least one, but generally two special moults from a number of different instars (reviewed by Roisin, 2000). However, in six species of

lower termites in two subfamilies (Termopsinae: *Archotermopsis wroughtoni* Desneux, *Zootermopsis angusticollis* (Hagen), *Zootermopsis nevadensis* (Hagen), *Zootermopsis laticeps* (Banks); Stolotermitinae: *Stolotermes brunneicornis* (Hagen), *Stolotermes ruficeps* Brauer), some neotenics may have soldier-like morphologies, and are called reproductive soldiers, or soldier neotenics (Myles, 1986). These soldier-like neotenics are phylogenetically rare, but have been found to be fairly common in some of the species in which they occur (Imms, 1919; Thorne et al., 2003). Soldier neotenics possess large mandibles similar to those of normal soldiers but have fully developed gonads (Heath, 1928; Castle, 1934b; Myles, 1986) and often exhibit behavior more similar to a reproductive than a soldier (Heath, 1928; Thorne et al., 2003). Soldier neotenics or “reproductive soldiers” are considered evolutionary relicts of an intermediate form of soldiers: a stepping-stone on the way to obligatory sterility and altruistic defense (Myles, 1986; Thorne et al., 2003).

Development of a soldier neotenic may increase a colony’s chance of survival and victory in the inevitable intercolony interactions faced by all primitive one-piece nesting termites (Thorne et al., 2003). Previous research demonstrated that soldier neotenics are effective at assassinating opposing primary reproductives of the same sex in intercolony conflicts (Thorne et al. 2003). No data yet exist on aggression of soldier neotenics towards normal neotenics in neighboring colonies, a dynamic critical for analyzing the costs and benefits of the production of each in the context of intercolony conflicts. Understanding the social and ecological conditions under which each type of neotenic reproductive develops, especially the unusual and

apparently primitive soldier neotenic, will illuminate selective pressures driving sterile and reproductive developmental pathways. Context influencing neotenic development will ultimately provide some insight into the evolution of castes and eusociality in this termite family.

Methods

I paired eight colonies that were headed by at least one neotenic in intercolony interactions (Table 7), mimicking the natural circumstances under bark. Interaction methodology was similar to those employed by Thorne et al. (2002). The colonies had been initiated from outbred alates in the spring of 2003 and were five and a half to six years old at the time of interaction. One colony participating in the interaction had at least one male normal neotenic, and the other had a male soldier neotenic. (I found male soldier neotenic to be much more common than female soldier neotenic (see Chapter 3), in contrast with some previous work (Castle, 1934b).) Paired colonies also had similar overall population sizes. I marked all individuals from each colony, aside from eggs and ‘larvae’³ (instars I-III), with Testor’s paint on the thorax or abdomen to denote their colony origin: one colony’s members were painted blue, the other’s red. I marked reproductive individuals with an additional unique dot of paint or two to serve as an individual identifier.

³ “larvae” is a misnomer because termites are hemimetabolous and all immatures are, according to normal entomological terminology, nymphs. However, “larvae” is used by convention to refer to I-III termite instars to differentiate them from pre-alate nymphs (reviewed in Thorne, 1996).

Table 7: Colony demographics for each interaction pair of Z.

***nevadensis* colonies**

	Interaction 1		Interaction 2		Interaction 3		Interaction 4	
Colony	A	B	A	B	A	B	A	B
Male reproductives	1 SN	1 N	1 SN	2 N	1 SN	2 N	1 SN	2 N
Female reproductives	7 N	2 N	5 N	2 N	3 N	6 N	2 N	2 N
Population	203	204	395	327	142	138	160	194
Soldiers	5	2	4	6	2	0	6	4
Larvae	0	0	2	0	43	4	0	42
Helpers	158	155	360	236	75	116	115	140
Nymphs	32	44	23	81	20	10	36	6

Table 7. Pre-interaction colony demographics for each interaction colony. “Soldier

neotenic” is abbreviated as “SN” and “neotenic” is abbreviated as “N.” Each colony in an interaction pair had either a soldier neotenic or neotenics of the same sex.

Colonies were also paired based on similar population sizes. Not included in the table are numbers of alates, which were removed from each colony prior to interaction.

I placed painted termites in custom-made clear plexiglass arenas (8 ½" width, 11" length, 1" inside depth) with loose-fitting lids of the same material. I clamped the lids down with large binder clips. These clear, flat dishes allowed for observation of action because they did not contain any large material that impeded viewing or allowed the termites to hide. I assigned one colony to each of two arenas that were connected by several centimeters of Tygon tubing inserted into pre-drilled holes in their sides. I clamped shut the tubing in the middle to close off access between the two colonies. I filled each dish with crumbled wood and feces from that colony’s nest and left the termites to habituate to this setting for one week.

After one week, I opened the connection between the colonies by removing the clamp on the tubing between them. For the next two hours, two observers recorded and videotaped all interactions between the two colonies. The newly merged colony remained in the interaction chamber overnight and was censused the next day. Following that census, I placed all individuals from the merged colony in a single container along with both sets of nesting materials.

I censused merged colonies the day after and a month after the interaction. To track persistence of individual reproductives, I gave each new reproductive appearing in the colony a uniquely-colored dot of Testor's paint on its thorax or abdomen. At each census I also re-painted older reproductives needing a paint touch-up in their original color.

Results

Helpers were almost always the aggressors in observed attacks on reproductive individuals (some attacks by nymphs were also observed). Helpers carried out almost all attacks on other helpers as well. The majority of termites in each colony were helpers (68-87%). All injured individuals were consumed, usually during the observation period but sometimes thereafter. In all cases in which I observed consumption, an injured termite was attacked and eaten by individuals from both colonies.

Normal soldiers were usually completely docile. I only saw biting by soldiers (against helpers or larvae) in interactions 1 and 4, though I observed occasional mandible flaring.

Nymphs (individuals with wing development) were usually docile as well. I observed one nymph biting an already-injured neotenic in interaction 1.

I saw no aggression by reproductives (neotenic or soldier neotenic), other than mandible flaring, towards any termite.

In three of the four interactions (#1, 2, and 4), all reproductives from the colony that had only neotenics were gone (consumed) 24 hours after mixing, while all reproductives from the soldier neotenic's colony survived through the first month after the interaction. Of these, new neotenics and soldier neotenics joined the existing reproductives as early as the first month after the interaction in trials 2 and 4, while interaction 1 had no new reproductives differentiate in addition to the pre-existing male soldier neotenic and seven female neotenics.

In one of four interactions (#3), all reproductives were dead after 24 hours. One month after the interaction, nine new neotenics of both sexes had developed (2 males, 7 females), as well as 5 pre-soldier neotenics, four males and one female. (The prefix "pre" refers to the fact that their exoskeleton had not yet sclerotized and they retained the white color of a newly-moulted individual.)

In interactions 1 and 2, most fighting occurred on the side of the chamber housing the original colony nest of the colony headed only by neotenics. In interactions 3 and 4, fighting occurred on both sides.

Most termites survived the interactions (Fig. 17). Termite survival by colony of origin is not included in survivorship data because many individuals lost their paint dot by the day after the interaction and colony of origin could no longer be determined.

Figure 17: Population Change After Interaction Between *Z.*

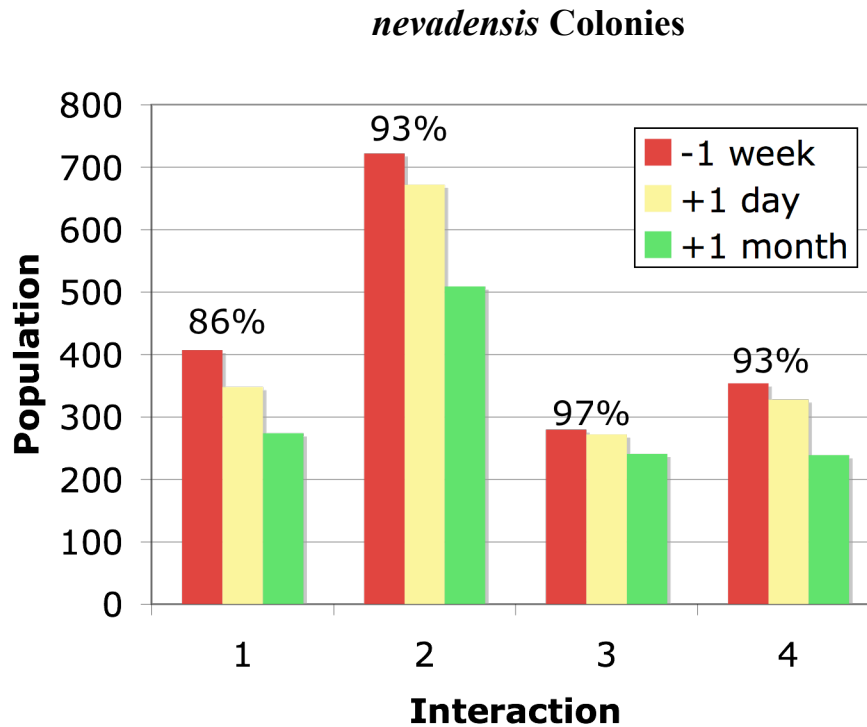


Figure 17. Pre- and post-interaction population counts for each interaction. Each of the 3 colors refers to a census: the week before the interaction (sum of the two individual colonies), the day after (merged colony), and a month after (merged colony). Percentages indicate survivorship from a week before the interaction to the day after the interaction. Interactions 1, 2, and 4 had no eggs in the +1 day census; this may account for their precipitous population declines to the +1 month census.

Discussion

There was a trend toward post-interaction survival of the soldier neotenic and other reproductives from its colony. It is unclear why the soldier neotenic and his related neotenics survived the interactions while the reproductives (all neotenics) from the

other colony were immediately killed. I observed no aggression from any reproductive, other than mandible flares by the soldier neotenics. Soldier neotenics may be somewhat protected by their more-armored head; however, this does not explain the consistent survival of normal neotenics within the soldier neotenic's colony. Preliminary observations suggest this survival pattern may change over time. In interaction 2, the surviving male soldier neotenic was absent 2 months post-interaction. Additional longer-term observations of all merged colonies will be necessary to elucidate survivorship of soldier neotenics through time.

Results of this experiment are consistent with the Accelerated Inheritance Hypothesis (Thorne et al., 2003). In three of four interactions, new reproductives differentiated in the month after the interaction, either to replace dead reproductives or to supplement the survivors. This suggests that in lower termites with flexible development and frequent intercolony interactions, there is a reasonable chance that helper termites will have opportunities to inherit the nest following reproductive loss. This might have been an important ecological driver of the evolution of eusociality in the Isoptera (Wilson, 1971; Alexander et al., 1991; Thorne, 1997, Thorne et al., 2003, Thorne & Traniello, 2003; Korb, 2008, Korb and Heinze, 2008).

In contrast with previous work with *Z. nevadensis* (Thorne et al., 2003), this experiment did not reveal an aggressive or “assassin” role for soldier neotenics in intercolony interactions. Soldier neotenics did not attack opposing-colony neotenics—or any termites. Their behavior during interactions was similar to that of

normal neotenic. This suggests that either they fail to recognize neotenic as reproductives, or do not display the same aggressive behavior towards neotenic reproductives as they do toward primary reproductives. These results also suggest that the possession of large, soldier-like mandibles does not necessarily make a neotenic termite more able to defend itself, although they may serve as a deterrent towards attacks.

Aggression among termite workers and helpers towards conspecific individuals from other colonies—as seen in this experiment—has been well-documented in a variety of taxa (e.g. Nel, 1968, Thorne, 1982, Haverty and Thorne, 1989, Polizzi and Forschler, 1998, Delphia et al., 2003). However, most termites in the colony were uninvolved in the interaction and survived, which is consistent with previous experiments with *Z. nevadensis* (Thorne et al., 2003). The relative docility of termite soldiers compared to the worker caste in these sorts of interactions has also been previously documented in various species (reviewed by Thorne, 1982). The limited interaction results presented here differ from recent work with an unrelated primitive one-piece nesting termite, the Kalotermitid *Cryptotermes secundus*, which found that soldiers are the key success predictor in intraspecific competition (Korb, 2008); in my work soldiers did not seem to participate in interaction outcomes. However, further interactions are needed to confirm this preliminary result.

Aside from the low sample size, several factors should be taken into consideration when interpreting data from this experiment. First, because the colonies used were

natural units raised in a lab for several years, they varied in a number of ways: relative caste proportions, genetic makeup, total number of reproductives, nutritive state, etc. The most important matching consideration I used, besides neotenic type, was population size, as previous research has shown that a much larger colony will destroy a smaller colony instead of merging (Thorne et al., 2002). Because these interactions occurred in the lab rather than in the field, some factors and stimuli that could affect aggression may not have been present, including, but not limited to previous interactions or the presence of ants or other predators. Additionally, although colonies were allowed to acclimate to their experimental interaction dishes for one week prior to interacting, they were still often noticeably agitated at the beginning of the interaction due to necessary handling of the dishes. This agitation may have affected the termites' behavior.

With combined colony sizes ranging to more than 700 individuals, it was impossible for two observers to keep track of all behaviors occurring in the interaction chamber. The observers focused their attention on the reproductive individuals as much as possible. However, as there were as many as a dozen or more reproductives, it was difficult to track all of them concurrently, even with video assistance. Because the ethological data were so similar between interactions, however, I feel that significant omissions were not likely.

Finally, laboratory rearing has been shown to decrease aggression in some termites (Nel, 1968). Because these termites were several generations removed from field-

collected individuals, their behaviors may be distorted. Furthermore, cuticular hydrocarbon profile is known to significantly affect kin recognition—and therefore aggression—between *Termopsid* colonies (e.g. Haverty and Thorne 1989; Delphia et al. 2003), although other factors also play a significant role (e.g. Adams, 1991; Dronnet et al., 2006; Adams et al., 2007). In this experiment, hydrocarbon profile was not taken into account in colony pairings, and colonies sometimes shared some distant ancestry. Because cuticular hydrocarbon similarity is correlated with genetic similarity (Dronnet et al., 2006), relatedness may have made individuals less aggressive than they would have been with completely unrelated termites. However, every interaction resulted in termites behaving aggressively towards termites of the other colony, suggesting that at least some individuals within each original colony were able to discriminate colony-mates from termites in the other colony. Furthermore, some research has suggested that relatedness between colonies is not an important predictor of aggression, but that intracolony relatedness is (Adams et al., 2007). Because all of the colonies used in this experiment were outbred, intracolony relatedness was consistent across all interactions and should not have skewed the outcomes observed.

Despite these caveats, results of this experiment offer further insight into caste roles during intercolony interactions in the evolutionarily important *Termopsids*, and will be continued with a larger sample size. Because these intercolony interactions are so ubiquitous in the life-history of primitive termites, understanding more about their

circumstances and results may shed light on the ecological conditions in which eusociality evolved in termites.

Appendix

Appendix

Figure 18: Relationship between Testis Width and Head Length

in Male Soldier Morphs of *Z. nevadensis*

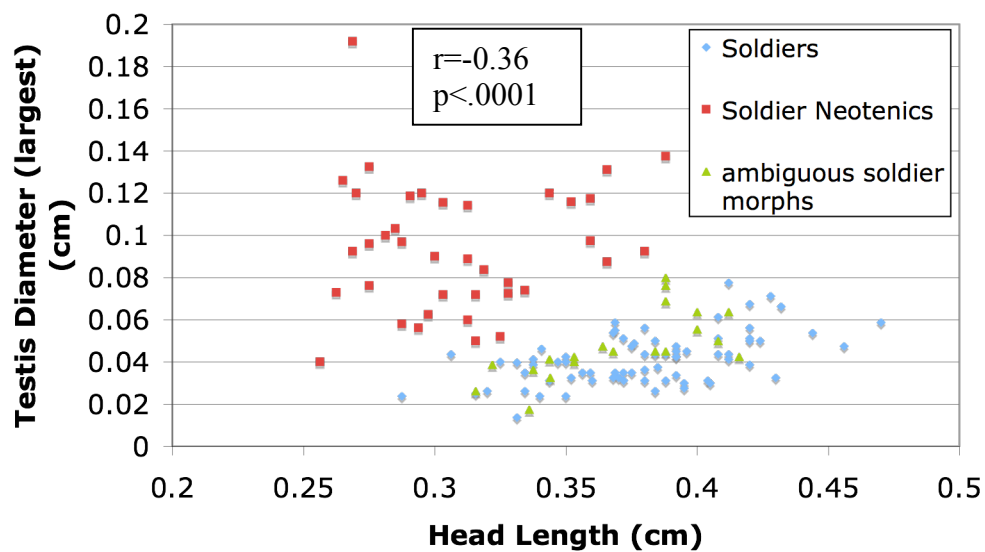
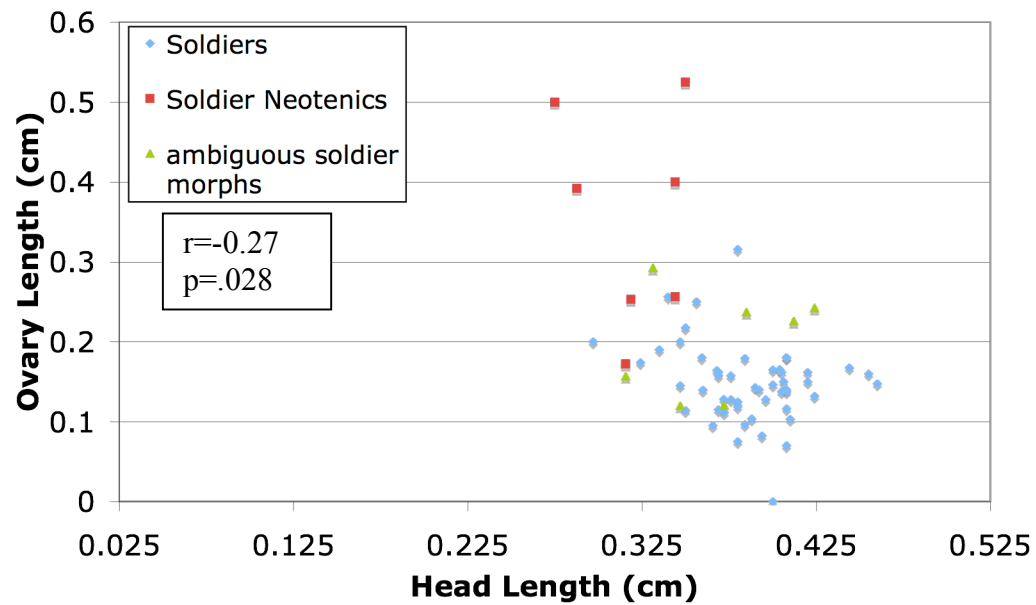


Fig. 18. Correlation between testis width (at widest point across) and head length in male soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

**Figure 19: Relationship between Ovary Length and Head Length
in Female Soldier Morphs of *Z. nevadensis***



**Figure 20: Relationship between Ovary Width and Head Length
in Female Soldier Morphs of *Z. nevadensis***

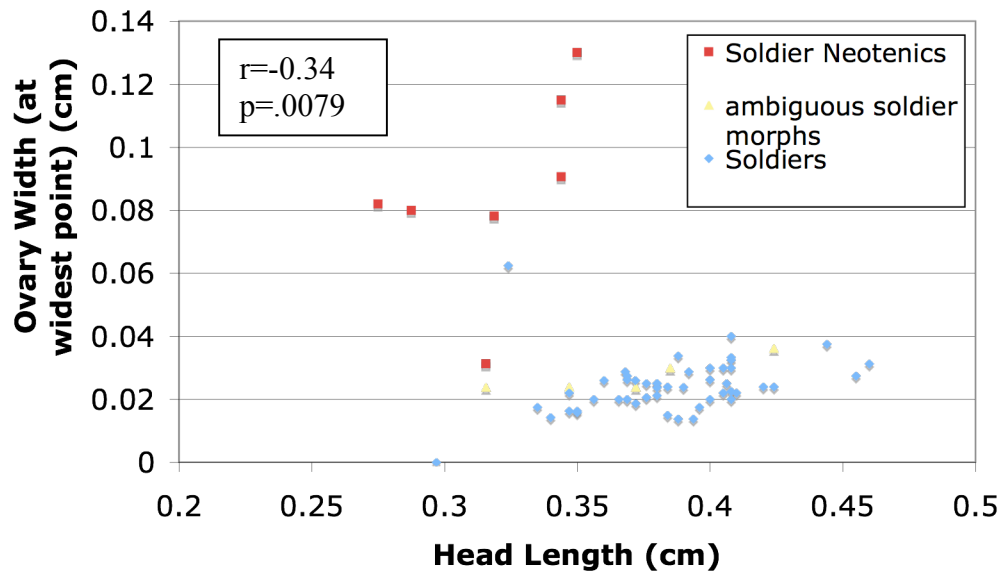


Fig. 20. Correlation between ovary width (at widest point across) and head length in female soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

**Figure 21: Relationship between Testis Width and Head Width
in Male Soldier Morphs of *Z. nevadensis***

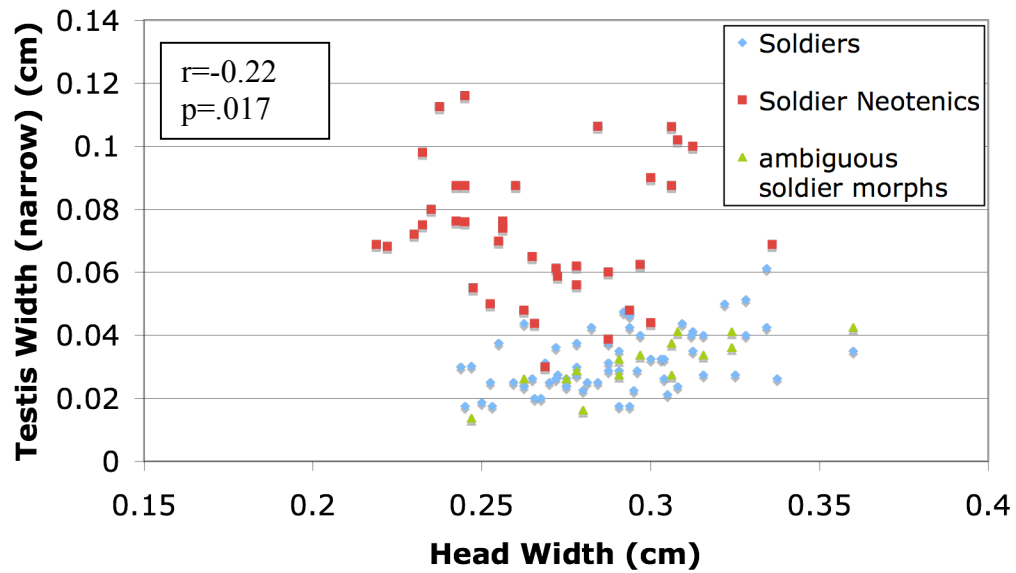


Fig. 21. Correlation between testis width (at narrowest point across) and head width in male soldier morphs (soldiers and soldier neoténics) of *Z. nevadensis*.

Figure 22: Relationship between Testis Width and Head

“Roundness” in Male Soldier Morphs of *Z. nevadensis*

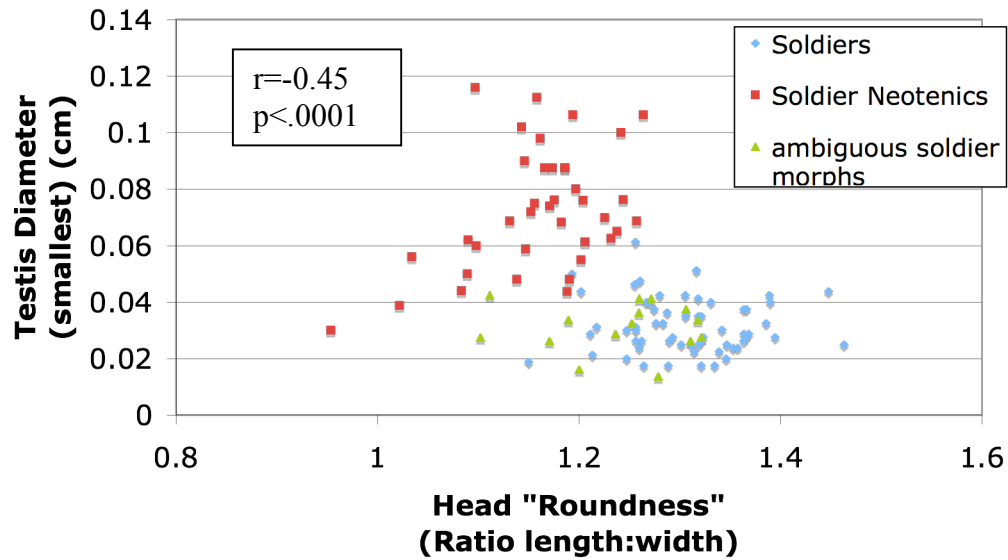


Fig. 22. Correlation between testis width (at narrowest point across) and head “roundness” (ratio of length to width) in male soldier morphs (soldiers and soldier neoténics) of *Z. nevadensis*.

**Figure 23: Relationship between Testis Width and Head
“Roundness” in Male Soldier Morphs of *Z. nevadensis***

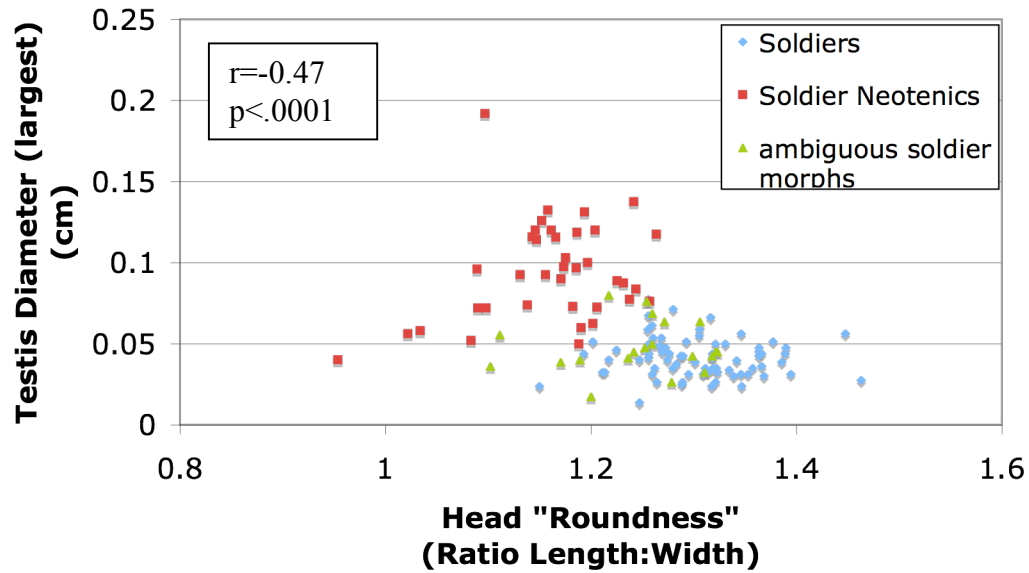


Fig. 23. Correlation between testis width (at widest point across) and head “roundness” (ratio of length to width) in male soldier morphs (soldiers and soldier neoténics) of *Z. nevadensis*.

**Figure 24: Relationship between Ovary Length and Head
“Roundness” in Female Soldier Morphs of *Z. nevadensis***

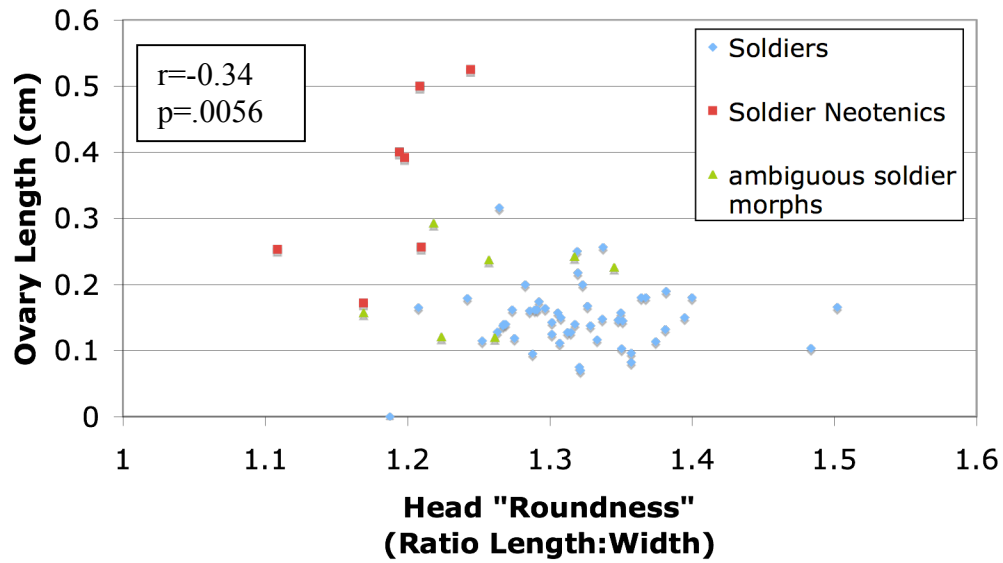


Fig. 24. Correlation between ovary length and head “roundness” (ratio of length to width) in female soldier morphs (soldiers and soldier neotenic) of *Z. nevadensis*.

Figure 25: Relationship between Ovary Width and Head “Roundness” in Female Soldier Morphs of *Z. nevadensis*

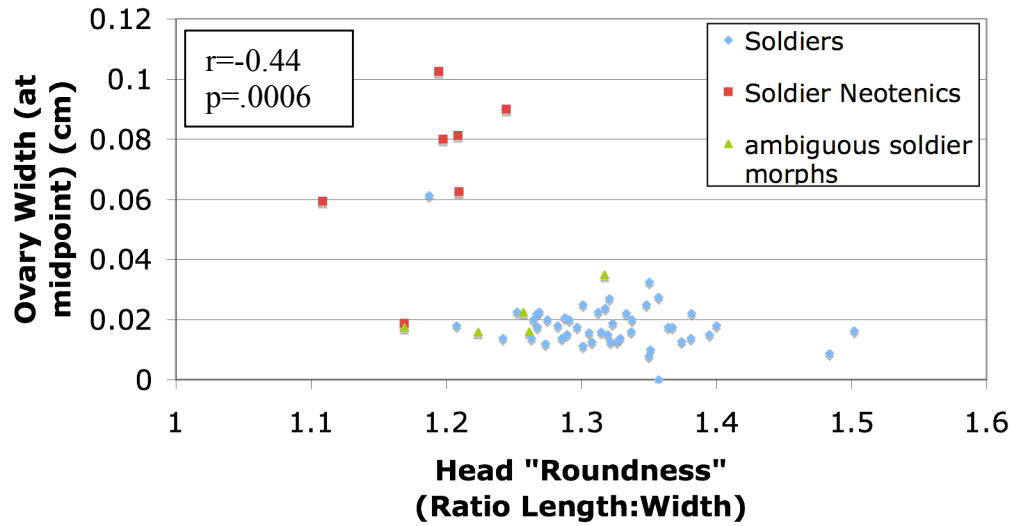


Fig. 25. Correlation between ovary width (at midpoint) and head “roundness” (ratio of length to width) in female soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

**Figure 26: Relationship between Ovary Width and Head
“Roundness” in Female Soldier Morphs of *Z. nevadensis***

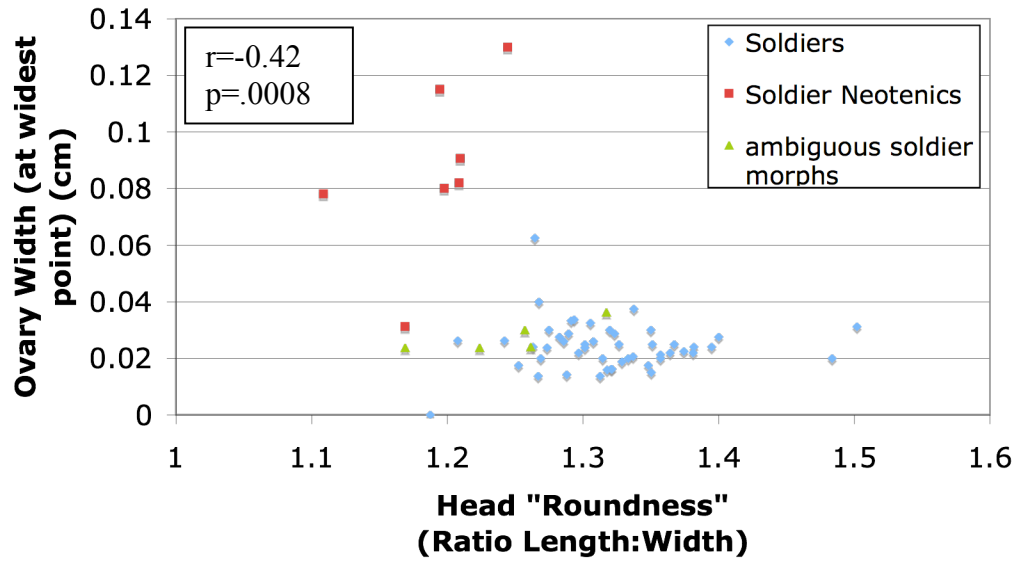


Fig. 26. Correlation between ovary width (at widest point across) and head “roundness” (ratio of length to width) in female soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

**Figure 27: Relationship between Testis Width and Mandible Length
in Male Soldier Morphs of *Z. nevadensis***

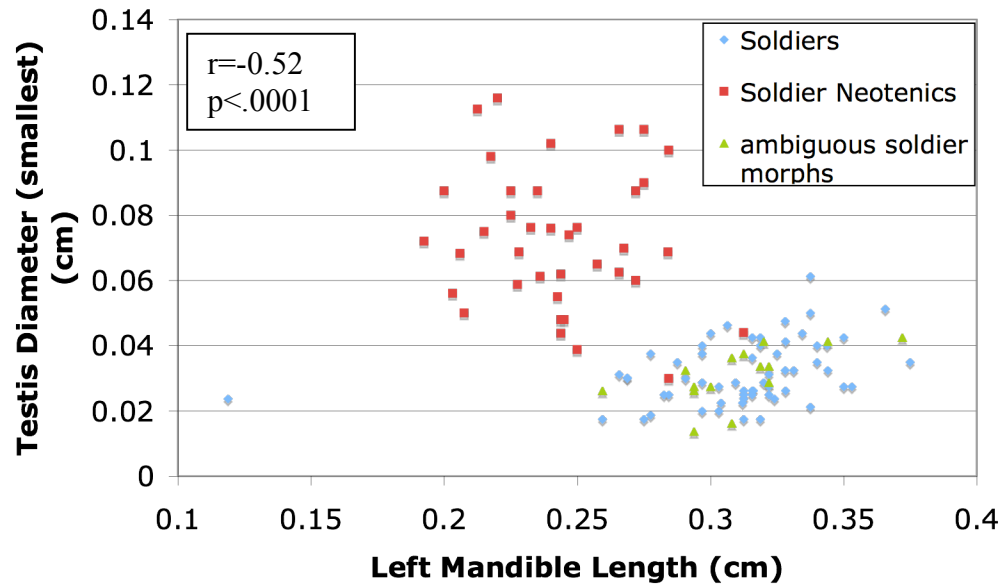
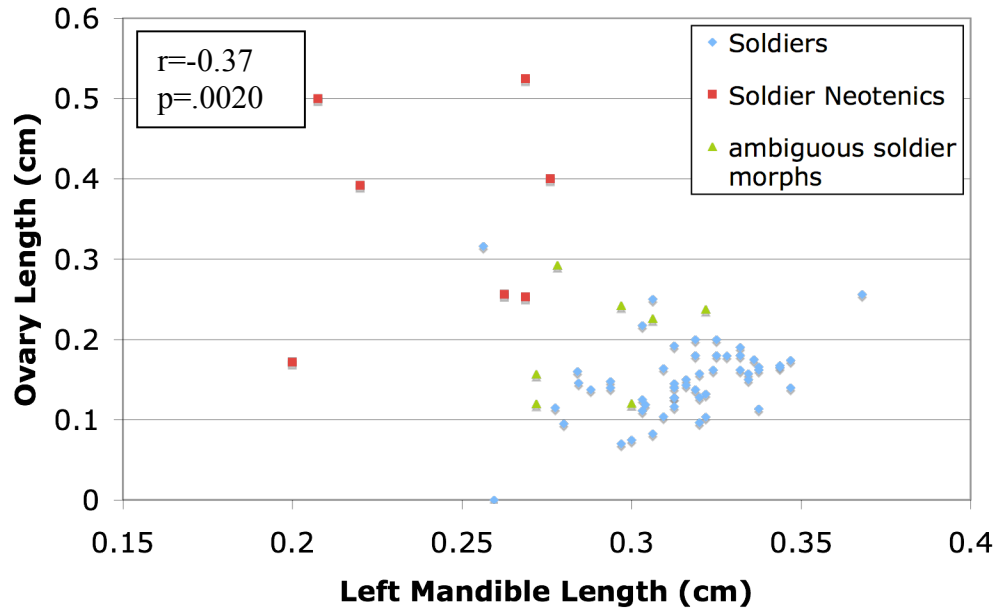


Fig. 27. Correlation between testis width (at narrowest point across) and left mandible length in male soldier morphs (soldiers and soldier neotenic) of *Z. nevadensis*.

Figure 28: Relationship between Ovary Length and Mandible

Length in Female Soldier Morphs of *Z. nevadensis*



**Figure 29: Relationship between Ovary Width and Mandible Length
in Female Soldier Morphs of *Z. nevadensis***

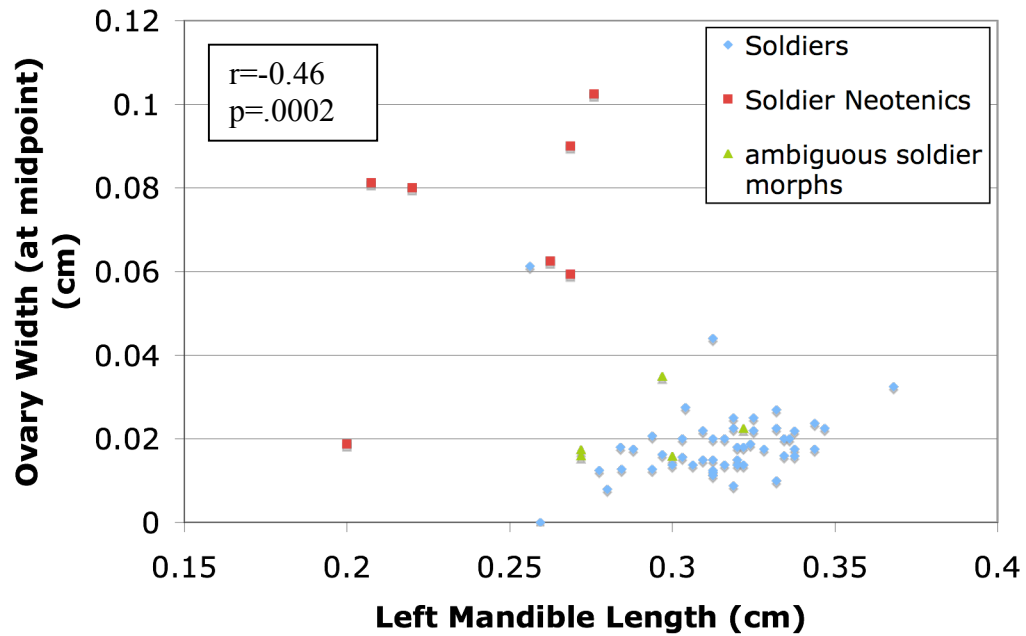


Fig. 29. Correlation between ovary width (at midpoint) and left mandible length in female soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

in Female Soldier Morphs of *Z. nevadensis*

female soldier morphs (soldiers and soldier neotenic) of *Z. nevadensis*.

in Female Soldier Morphs of *Z. nevadensis*

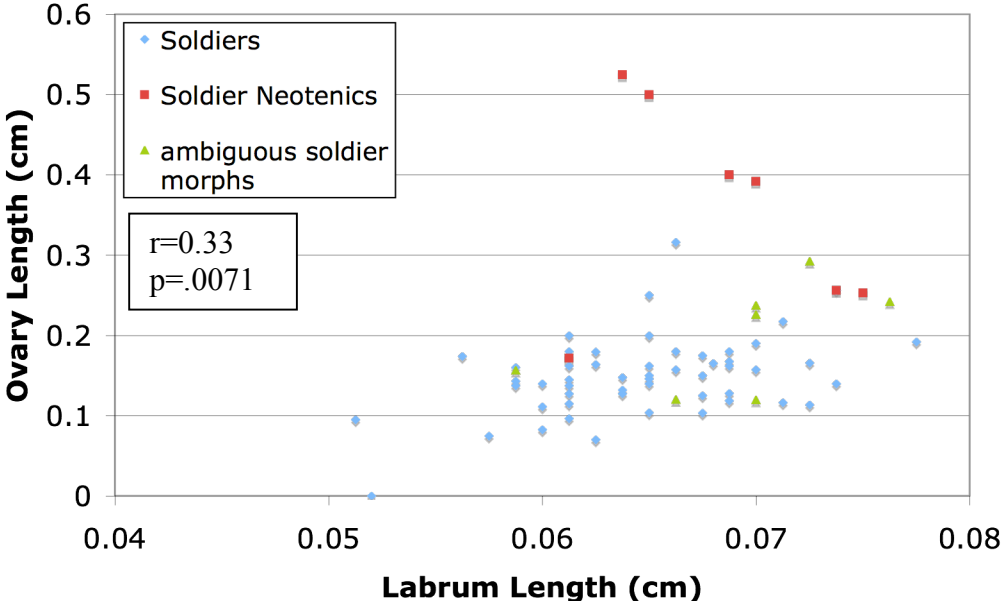


Fig. 31. Correlation between ovary length and labrum length in female soldier morphs (soldiers and soldier neotenic) of *Z. nevadensis*.

**Figure 32: Relationship Between Ovary Width (at midpoint)
and Labrum Length in Female Soldier Morphs of *Z. nevadensis***

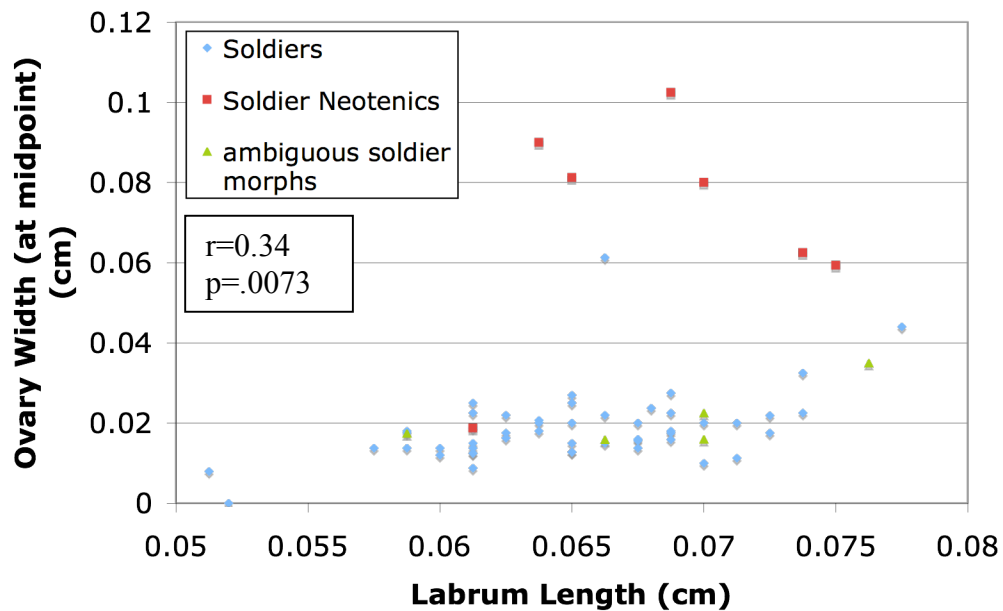


Fig. 32. Correlation between ovary width (at midpoint) and labrum length in female soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

Figure 33: Relationship between Ovary Width and Labrum Length
in Female Soldier Morphs of *Z. nevadensis*

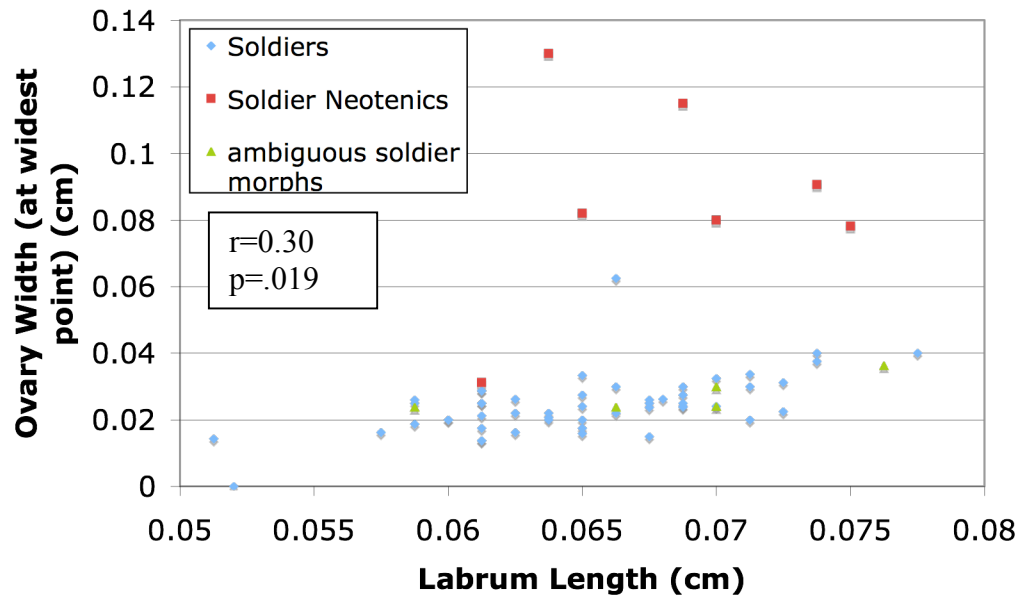


Fig. 33. Correlation between ovary width (at widest point) and labrum length in female soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

**Figure 34: Relationship between Testis Width and Labrum Width
in Male Soldier Morphs of *Z. nevadensis***

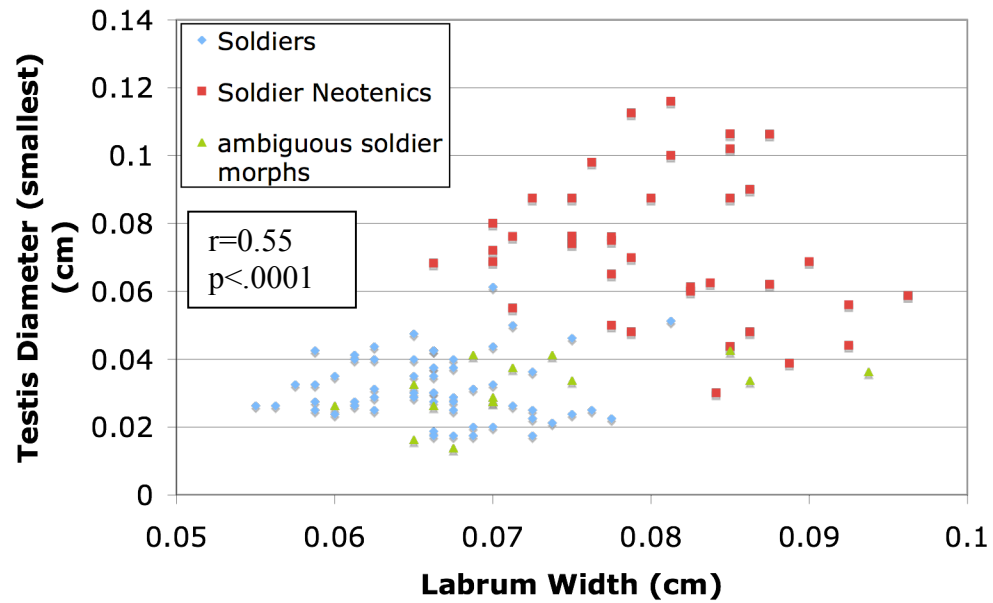


Fig. 34. Correlation between testis width (at narrowest point) and labrum width in male soldier morphs (soldiers and soldier neoténics) of *Z. nevadensis*.

**Figure 35: Relationship between Testis Width and Labrum Width
in Male Soldier Morphs of *Z. nevadensis***

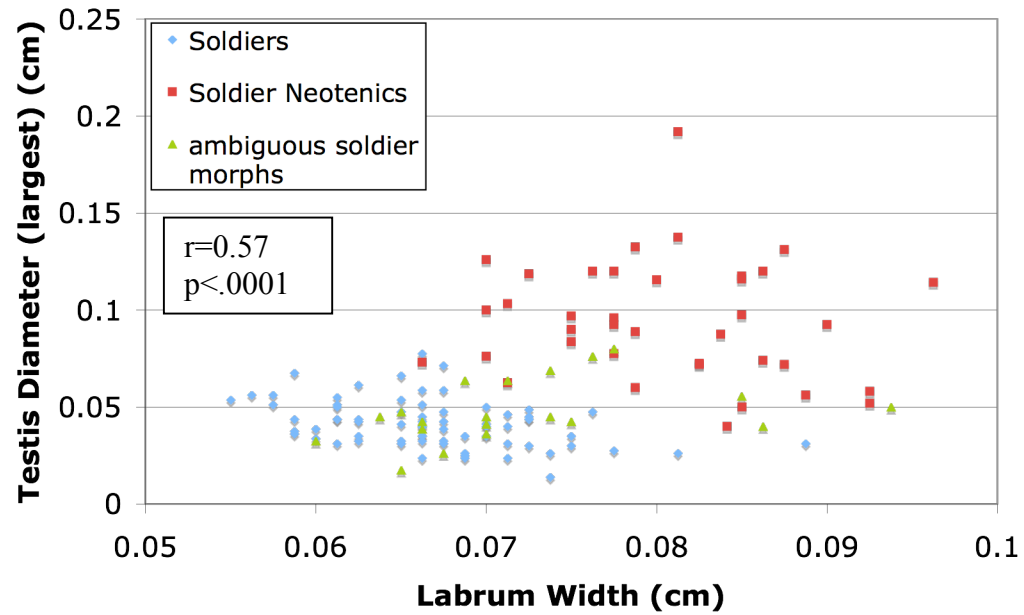


Fig. 35. Correlation between testis width (at widest point) and labrum width in male soldier morphs (soldiers and soldier neotenic) of *Z. nevadensis*.

Figure 36: Relationship between Ovary Length and Labrum Width
in Female Soldier Morphs of *Z. nevadensis*

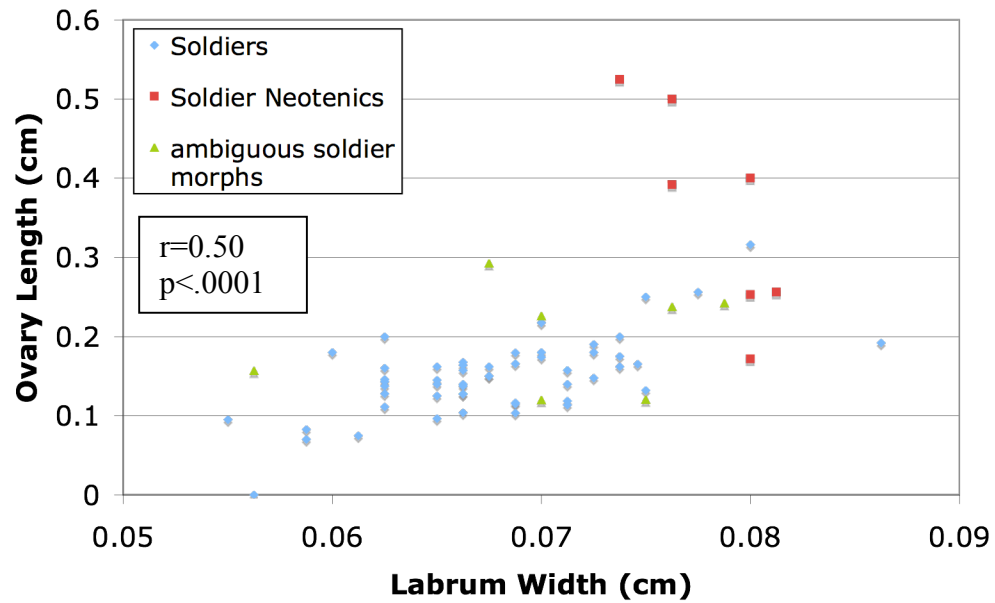


Fig. 36. Correlation between ovary length and labrum width in female soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

**Figure 37: Relationship between Ovary Width and Labrum Width
in Female Soldier Morphs of *Z. nevadensis***

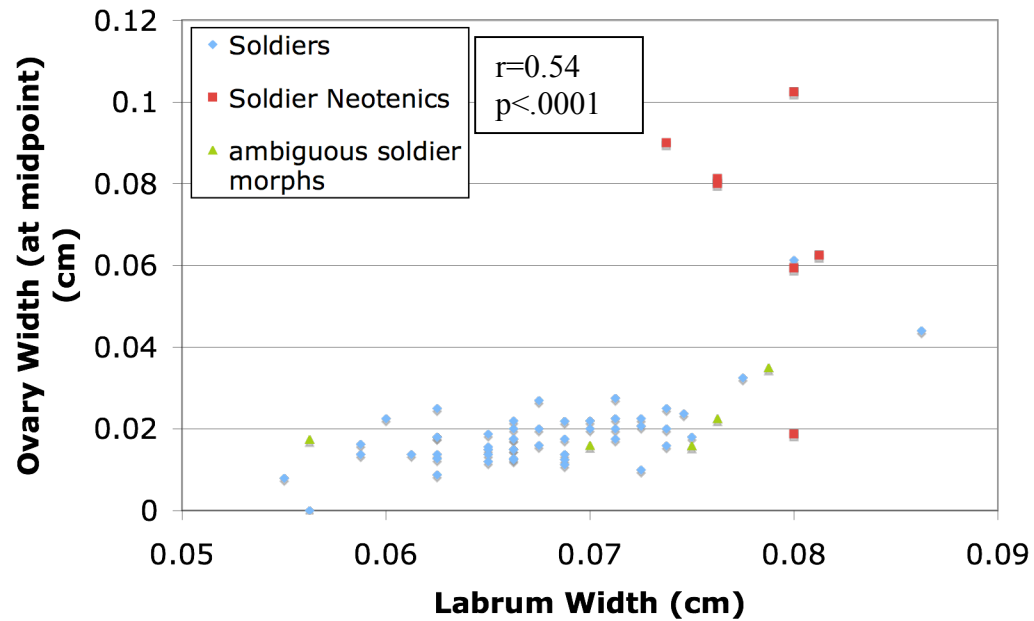


Fig. 37. Correlation between ovary width (at midpoint) and labrum width in female soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

**Figure 38: Relationship between Ovary Width and Labrum Width
in Female Soldier Morphs of *Z. nevadensis***

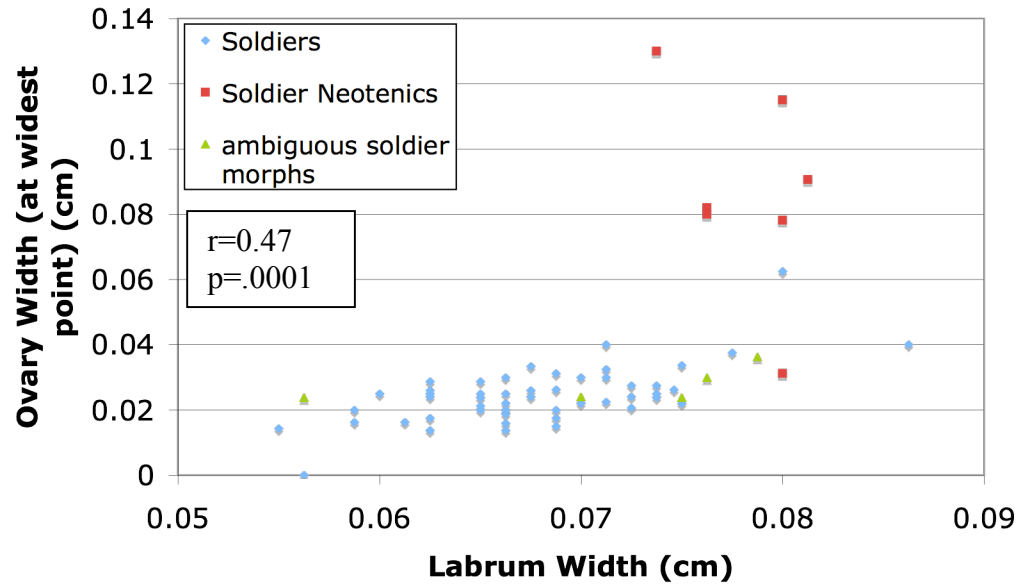


Figure 39: Relationship between Testis Width and Labrum

“Roundness” in Male Soldier Morphs of *Z. nevadensis*

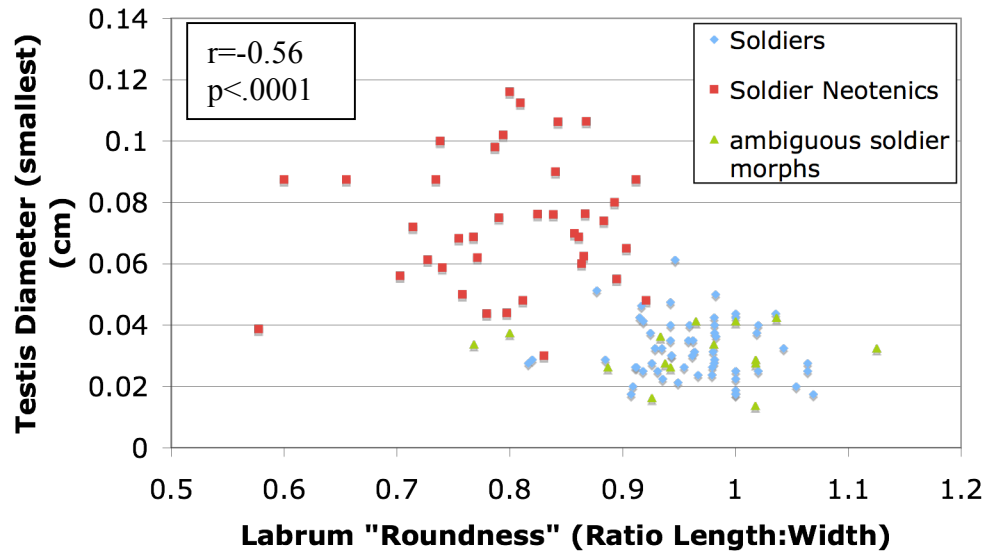


Fig. 39. Correlation between testis width (at smallest point across) and head “roundness” (ratio of labrum length to width) in male soldier morphs (soldiers and soldier neoténics) of *Z. nevadensis*.

Figure 40: Relationship between Ovary Length and Labrum

“Roundness” in Female Soldier Morphs of *Z. nevadensis*

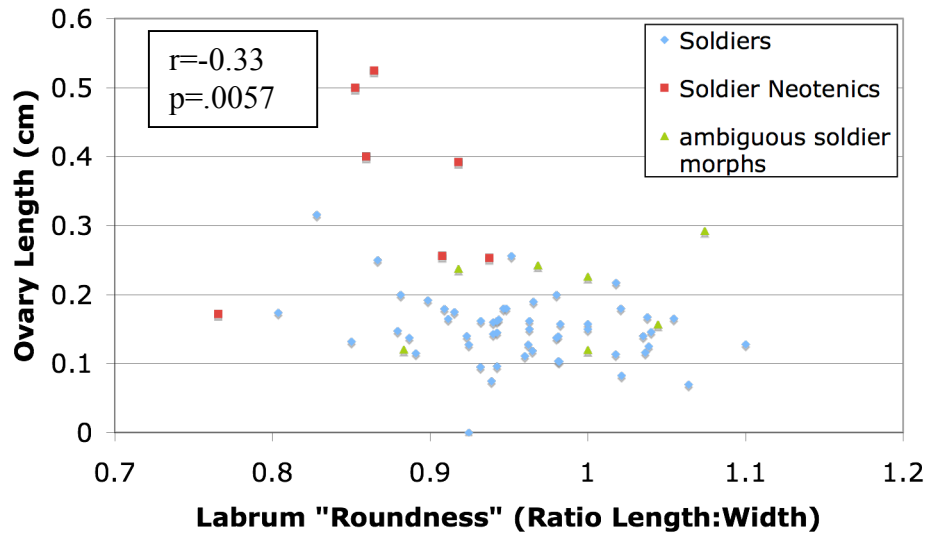


Figure 41: Relationship between Ovary Width and Labrum

“Roundness” in Male Soldier Morphs of *Z. nevadensis*

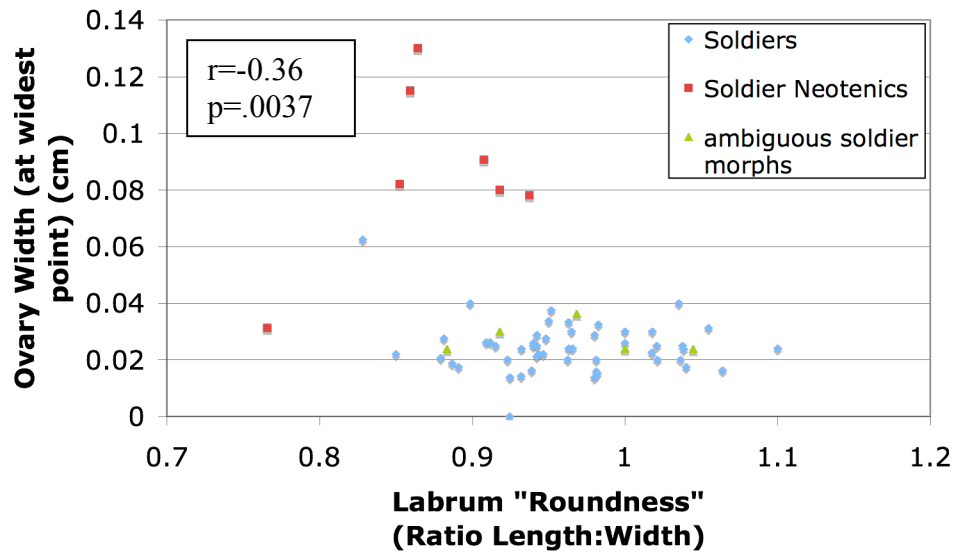


Fig. 41. Correlation between ovary width (at widest point) and labrum “roundness” (ratio of labrum length to width) in female soldier morphs (soldiers and soldier neoténics) of *Z. nevadensis*.

Figure 42: Relationship between Testis Width and Postmentum

Length in Male Soldier Morphs of *Z. nevadensis*

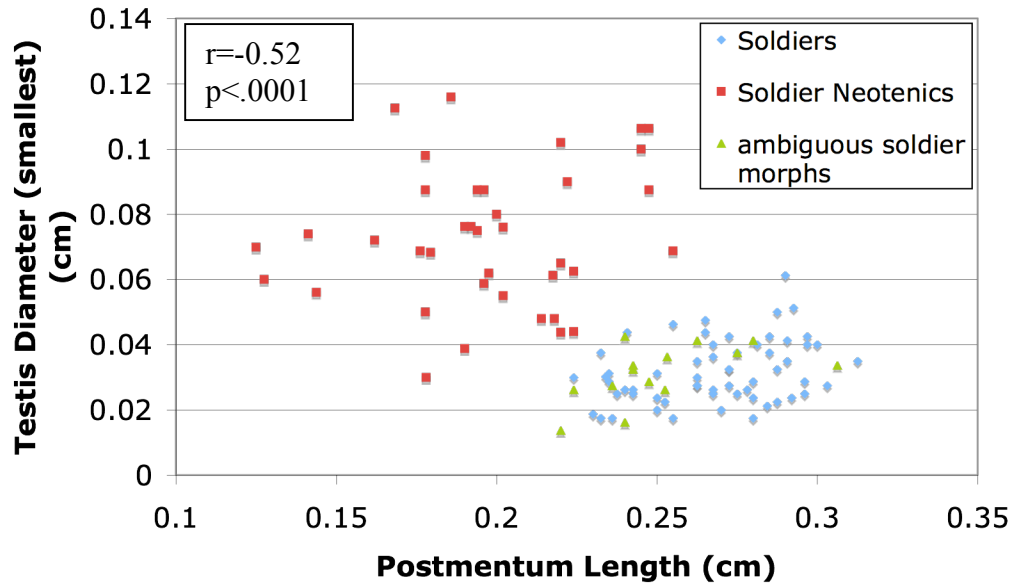


Fig. 42. Correlation between testis width (at smallest point across) and postmentum length in male soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

Length in Male Soldier Morphs of *Z. nevadensis*

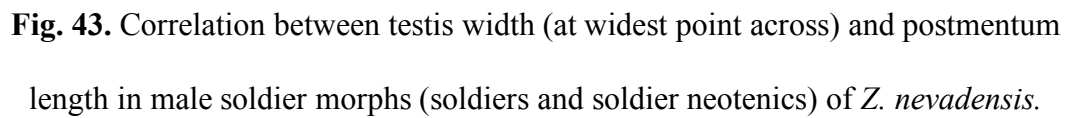


Figure 44: Relationship between Testis Width and Postmentum

Width (at widest point) in Male Soldier Morphs of *Z. nevadensis*

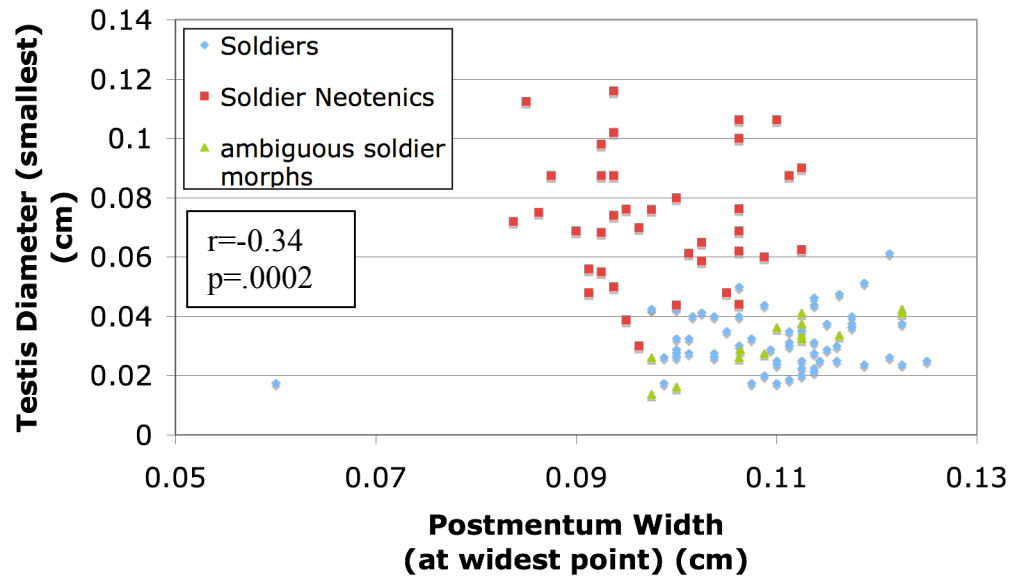


Fig. 44. Correlation between testis width (at narrowest point across) and postmentum width (at widest point across) in male soldier morphs (soldiers and soldier neotenic) of *Z. nevadensis*.

Figure 45: Relationship between Testis Width and Postmentum

Width (at widest point) in Male Soldier Morphs of *Z. nevadensis*

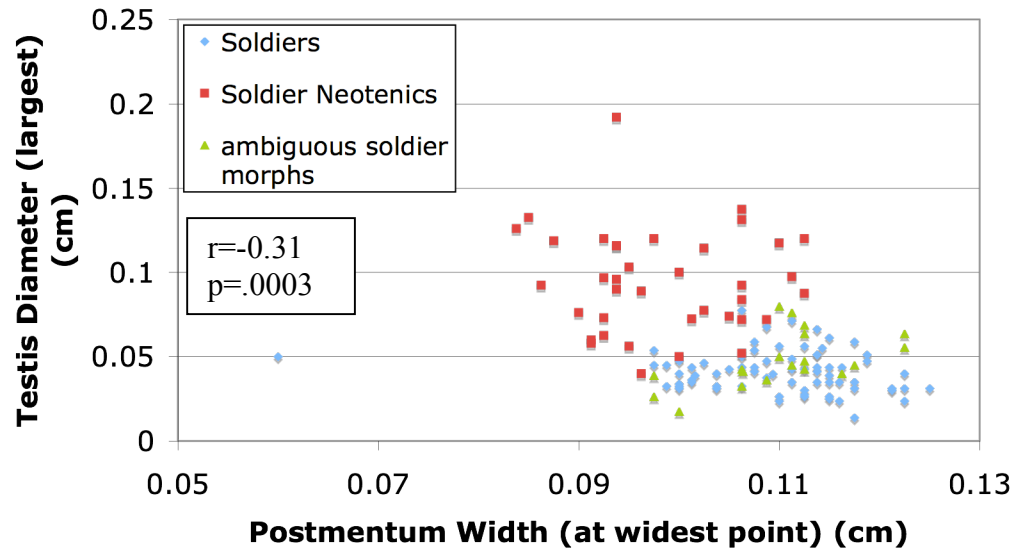


Fig. 45. Correlation between testis width (at widest point across) and postmentum width (at widest point across) in male soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

Figure 46: Relationship between Testis Width and Postmentum Width (at narrowest point) in Male Soldier Morphs of *Z. nevadensis*

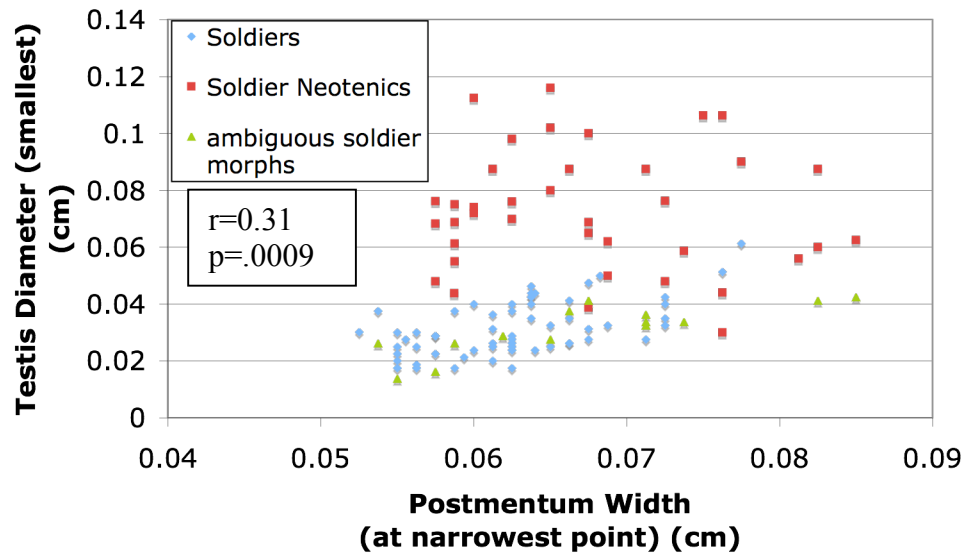


Fig. 46. Correlation between testis width (at narrowest point across) and postmentum width (at narrowest point across) in male soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

**Figure 47: Relationship between Testis Width and Postmentum Width
(at narrowest point) in Male Soldier Morphs of *Z. nevadensis***

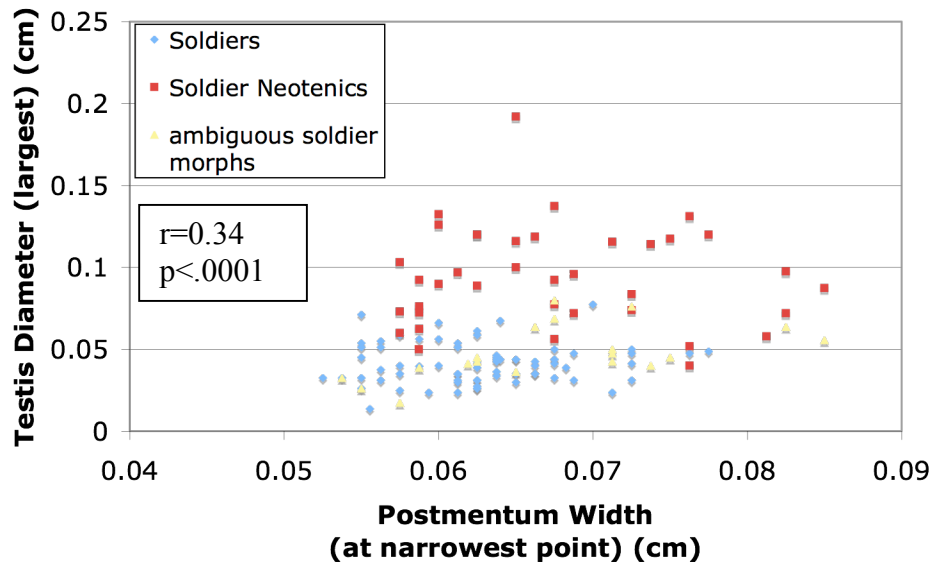


Fig. 47. Correlation between testis width (at widest point across) and postmentum width (at narrowest point across) in male soldier morphs (soldiers and soldier neotenic) of *Z. nevadensis*.

Figure 48: Relationship between Ovary Length and Postmentum

Width (at narrowest point) in Female Soldier Morphs of *Z.*

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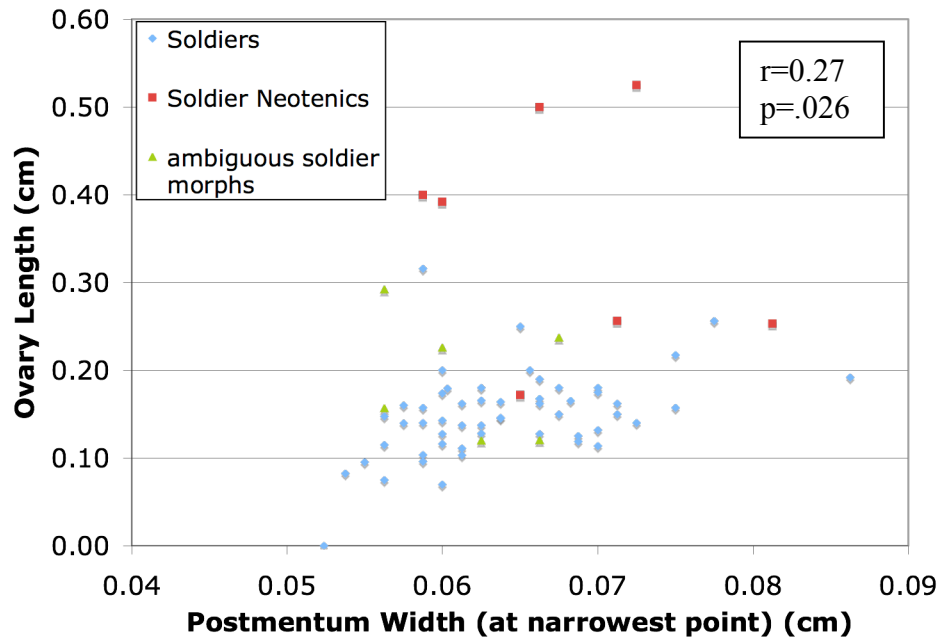


Fig. 48. Correlation between ovary length and postmentum width (at narrowest point across) in female soldier morphs (soldiers and soldier neotenic) of *Z. nevadensis*.

Figure 49: Relationship between Ovary Width and Postmentum

Width (at narrowest point) in Male Soldier Morphs of *Z. nevadensis*

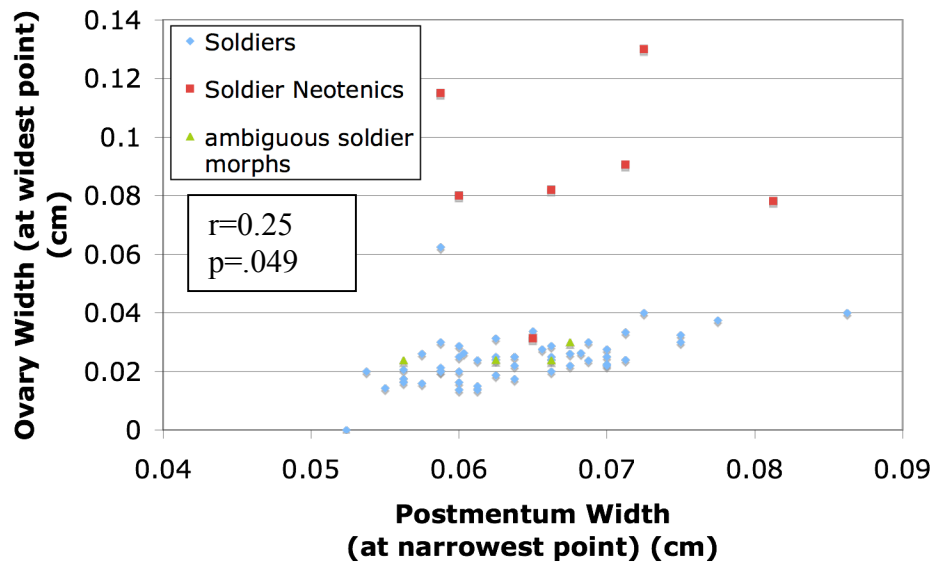


Figure 50: Relationship between Testis Width and Eye Length
in Male Soldier Morphs of *Z. nevadensis*

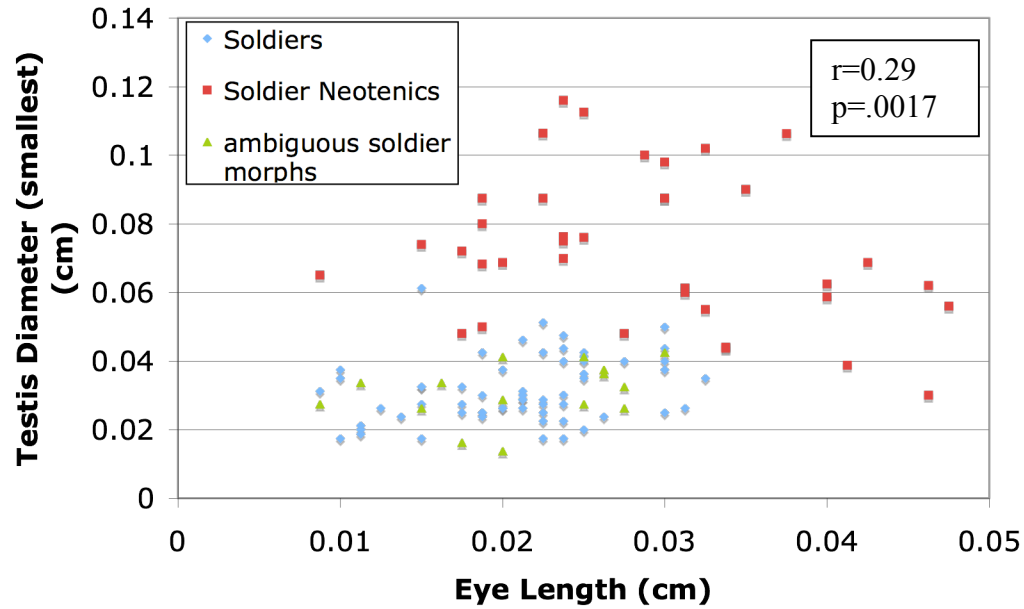


Fig. 50. Correlation between testis width (at narrowest point across) and eye length in male soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

**Figure 51: Relationship between Testis Width and Eye Length
in Male Soldier Morphs of *Z. nevadensis***

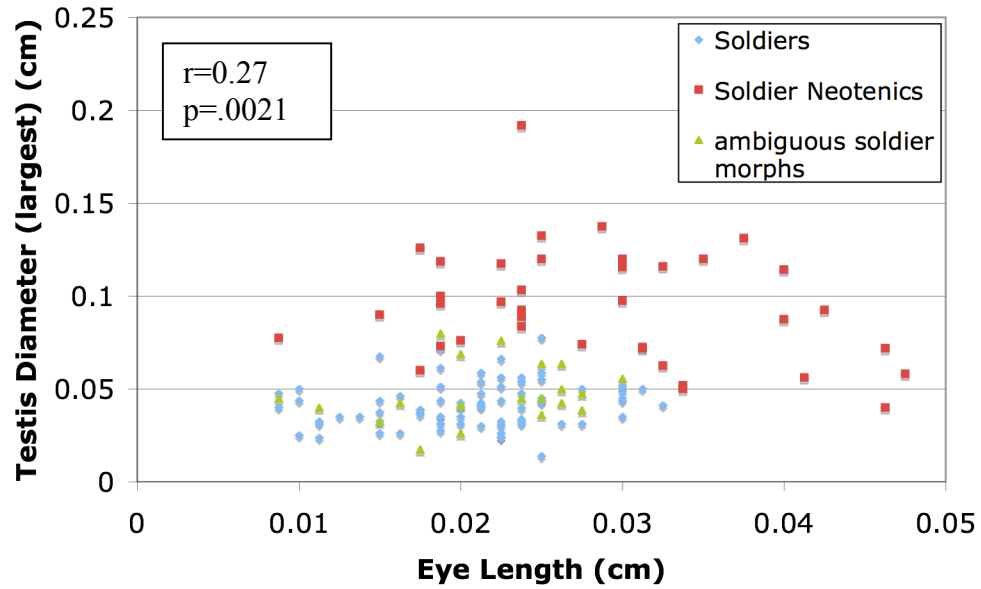
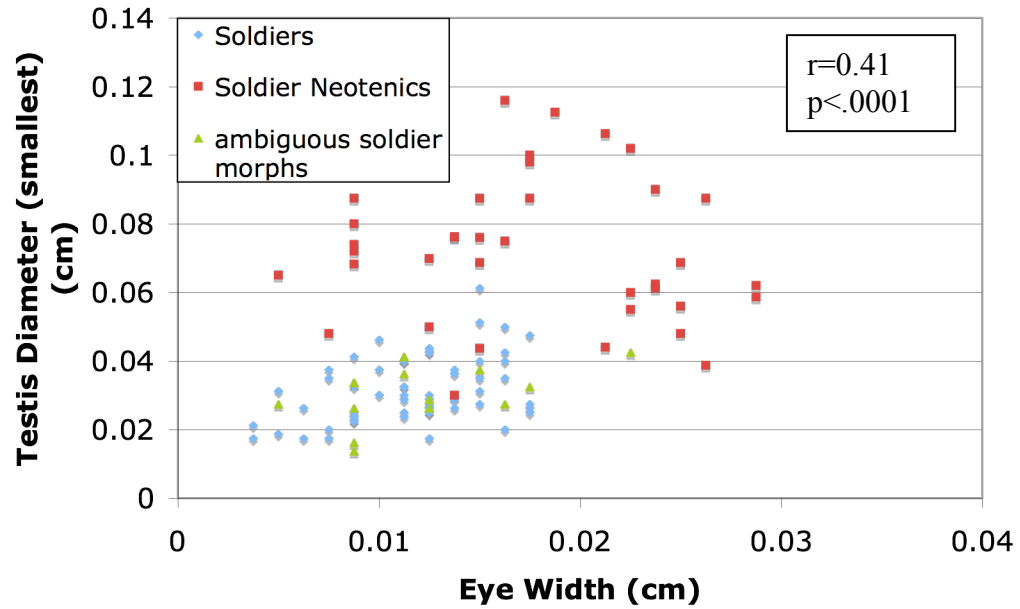


Fig. 51. Correlation between testis width (at widest point across) and eye length in male soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

**Figure 52: Relationship between Testis Width and Eye Width
in Male Soldier Morphs of *Z. nevadensis***



**Figure 53: Relationship between Testis Width and Eye Width
in Male Soldier Morphs of *Z. nevadensis***

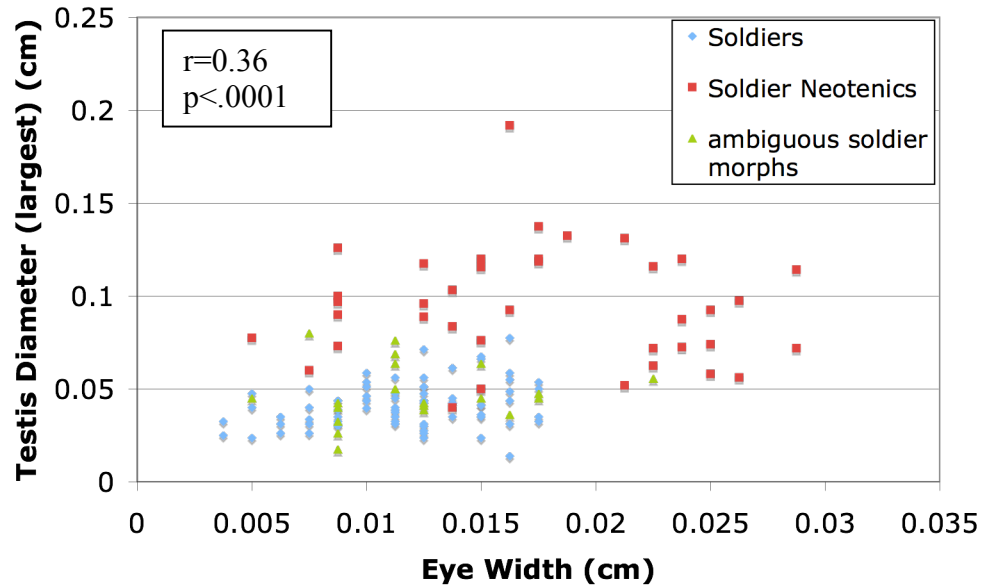


Fig. 53. Correlation between testis width (at widest point across) and eye width in male soldier morphs (soldiers and soldier neotenic) of *Z. nevadensis*.

**Figure 54: Relationship between Ovary Length and Eye Width
in Female Soldier Morphs of *Z. nevadensis***



Fig. 54. Correlation between ovary length and eye width in female soldier morphs
(soldiers and soldier neotenuics) of *Z. nevadensis*.

**Figure 55: Relationship between Ovary Width and Eye Width
in Female Soldier Morphs of *Z. nevadensis***

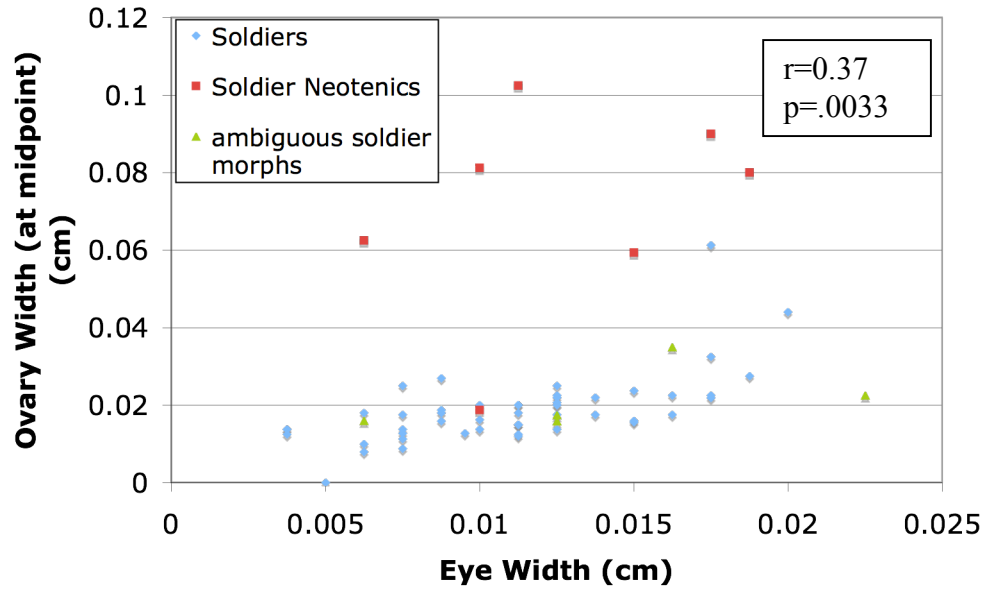


Fig. 55. Correlation between ovary width (at midpoint) and eye width in female soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

**Figure 56: Relationship between Ovary Width and Eye Width
in Female Soldier Morphs of *Z. nevadensis***

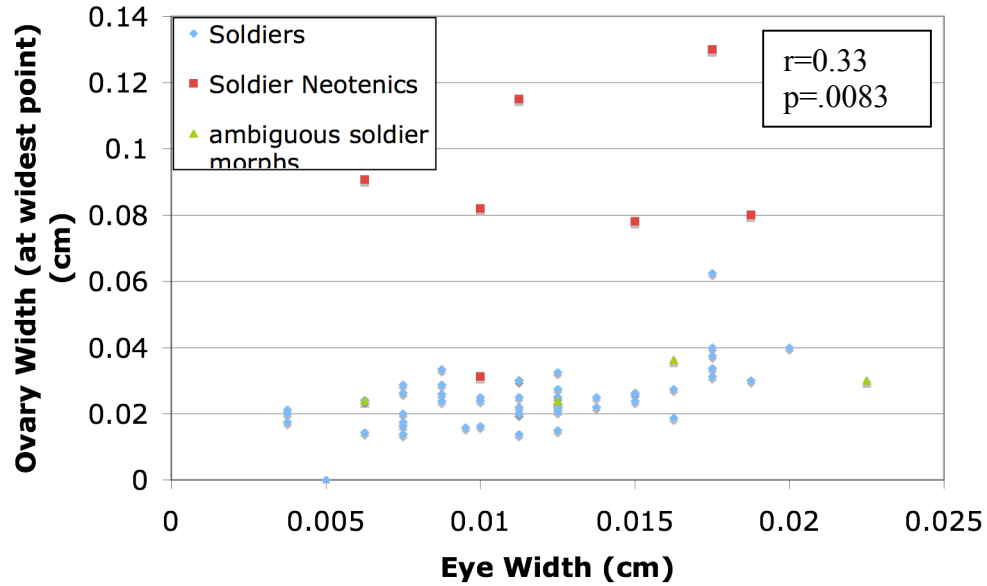


Fig. 56. Correlation between ovary width (at widest point) and eye width in female soldier morphs (soldiers and soldier neotenics) of *Z. nevadensis*.

Figure 57: Relationship between Testis Width and the Ratio of Mandible to Labrum Length in Male Soldier Morphs of *Z. nevadensis*

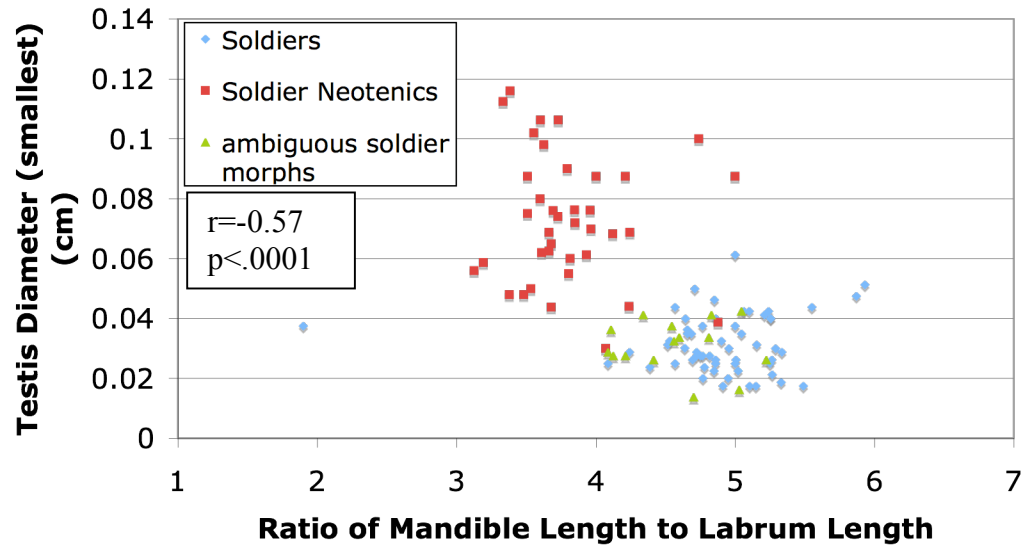


Fig. 57. Correlation between testis width (at narrowest point across) and the ratio of mandible length to labrum length in male soldier morphs (soldiers and soldier neoténics) of *Z. nevadensis*.

Figure 58: Relationship between Ovary Width and the Ratio of Mandible to Labrum Length in Female Soldier Morphs of *Z. nevadensis*

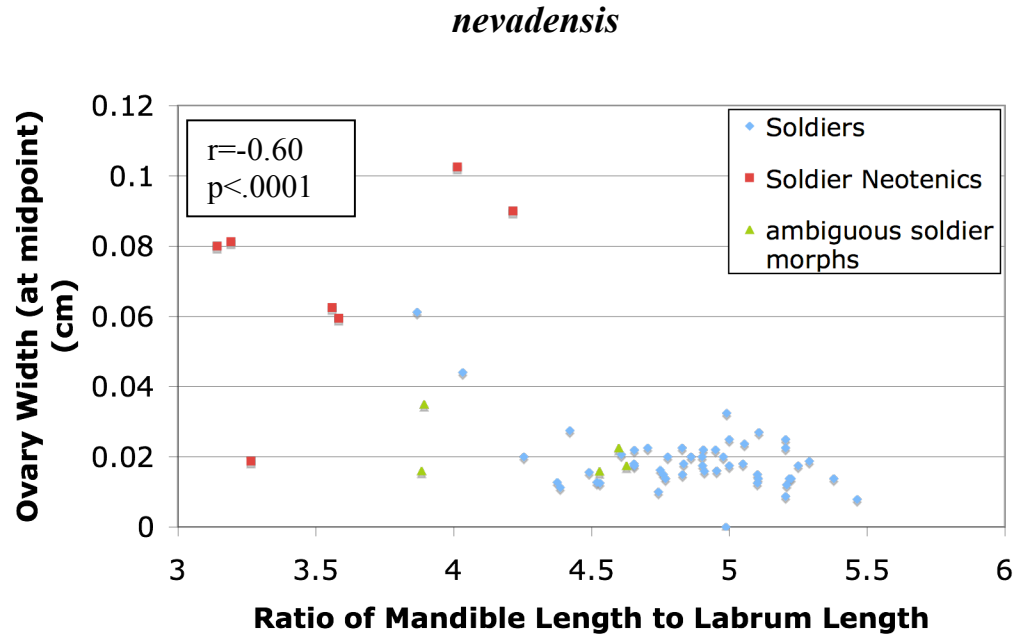


Fig. 58. Correlation between ovary width (at midpoint) and the ratio of mandible length to labrum length in female soldier morphs (soldiers and soldier neoténics) of *Z. nevadensis*.

Figure 59: Relationship between Ovary Width and the Ratio of Mandible to Labrum Length in Female Soldier Morphs of *Z. nevadensis*.

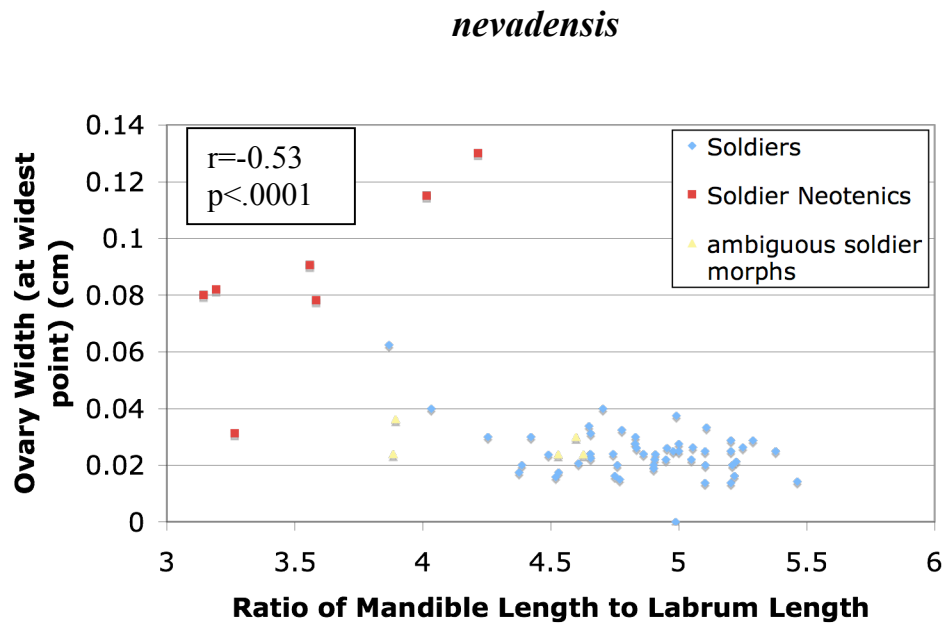


Fig. 59. Correlation between ovary width (at widest point) and the ratio of mandible length to labrum length in female soldier morphs (soldiers and soldier neoténics) of *Z.*

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