

# Selection on defensive traits in a sterile caste – caste evolution: a mechanism to overcome life-history trade-offs?

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**SUMMARY** During development and evolution individuals generally face a trade-off between the development of weapons and gonads. In termites, characterized by reproductive division of labor, a caste evolved—the soldiers—which is completely sterile and which might be released from developmental trade-offs between weapons and testes. These soldiers are exclusively dedicated to defense. First, we investigated whether defensive traits are under selection in sterile termite soldiers using allometric analyses. In soldiers of the genus *Cryptotermes* phragmotic traits such as a sculptured and foreshortened head evolve rapidly but were also lost twice. Second, we compared the scaling relationships of these weapons with those in solitary insects facing a trade-off between weapons and gonads. Defensive traits consistently had lower slopes than nondefensive traits which supports the

existence of stabilizing selection on soldier phragmotic traits in order to plug galleries. Moreover, soldier head widths were colony specific and correlated with the minimum gallery diameter of a colony. This can proximately be explained by soldiers developing from different instars. The scaling relationships of these termite soldiers contrast strikingly with those of weapons of solitary insects, which are generally exaggerated (i.e., overscaling) male traits. These differences may provide important insights into trait evolution. Trade-offs constraining the development of individuals may have been uncoupled in termites by evolving different castes, each specialized for one function. When individuals in social insect are “released” from developmental constraints through the evolution of castes, this certainly contributed to the ecological and evolutionary success of social insects.

## INTRODUCTION

Adaptive phenotypic plasticity can be defined as a genotype's ability to adjust patterns of phenotype expression to suit current or future environmental conditions (Via et al. 1995). Adaptive phenotypic plasticity is carried to extremes in polyphenic organisms. Here, individuals are able to express two or more discrete phenotypes in response to differences in the internal or external environment experienced by the developing organism (Nijhout 1999, 2003).

One of the most impressive examples of polyphenism are the castes of social insects. In termites, ants and some wasps and bees, the worker and reproductive caste generally develops from the same genotype depending on environmental and social stimuli (although exceptions can exist: e.g., Helms Cahán and Keller 2003; Hayashi et al. 2007). Additionally, defensive morphs occur. However, morphologically specialized castes dedicated to defense are rare, except in termites, and most seem to have evolved secondarily (ants: Baroni and Passera 1996; aphids: Stern and Foster 1997; thrips: Chapman

et al. 2002). Isopteran soldiers are unique in the eusocial world, for they (i) are monophyletic and ancestral to all termites and (ii) have an exclusive defensive function incompatible with reproduction and self-maintenance (Noirot and Pasteels 1987; Noirot 1990).

The proximate mechanisms that regulate polyphenic development in termite soldiers have been studied on a variety of levels, including gene expression, the endocrinological and the social scale (reviewed in Korb and Hartfelder 2008). However, studies on the evolutionary function are rare (Matsuura 2002; Roux and Korb 2004). There is a general agreement over the adaptive value of soldiers' defense (Noirot and Darlington 2000). The variety of soldier weapons (biting, phragmosis, and chemical secretion), together with the diversity of mandible morphologies (Deligne et al. 1981; Grassé 1982a; Prestwich 1984), tends to support this view. In the genus, *Cryptotermes*, the soldiers' main defense is achieved via phragmosis (i.e., plug shaped heads are used to stopper the galleries; sensu Brown 1967), mandibles are secondary and can even regress completely (Scheffrahn et al. 1998; Thomp-

son et al. 2000). Behavioral phragmosis is thought to be ancestral and widespread in termites.

Behavioral phragmosis is not restricted to termites and evolved independently in a variety of taxa (ants, toads, frogs, armadillos, snakes, spiders, and beetles). Morphological adaptations to phragmosis result in the modification of the head or the posterior end of the body for closing galleries, entrances to burrows or nests, and (in higher termites) internal connecting apertures between galleries (Wheeler 1927). For an effective blocking to be possible the dimensions of soldier heads must match the average gallery diameters as closely as possible. In termites, the galleries are excavated by workers, not soldiers, and in incipient colonies soldiers develop after a certain colony size has been reached (Noirot 1985), i.e., when a gallery system already exists. Thus, the facultative adjustment of galleries to soldier size seems unlikely and defense will be best achieved when the size of soldier heads is stabilized around the gallery diameter value.

The aim of this study was 2-fold: first, we tested whether phragmotic defense in soldiers of a true drywood termite (*sensu* Thompson et al. 2000), *Cryptotermes secundus* (Hill), is under stabilizing selection and second, we compared our results for sterile workers with those for weapons in other species in which individuals face a trade-off between investment in defense and reproduction. (*Note*: Workers are referred to as “false workers” in this study as they are immatures which retain the ability to develop into sexuals or soldiers [Korb and Katrantzis 2004] and because they are less engaged in helping to raise siblings [Korb 2007].) Regarding our first aim, several observations suggested that phragmotic defense might no longer be selected for. (i) A phylogenetic study of the Australian drywood termites revealed that phragmotic traits, sculptured and foreshortened heads, have evolved rapidly but were also lost twice independently (Thompson et al. 2000). (ii) Behavioral observations showed that soldiers in *C. secundus* do not have a highly specialized defensive behavior compared with old false workers; both defend by biting with their mandibles (but phragmosis was not observed, Roux and Korb 2002). (iii) Although we could show that *C. secundus* soldiers gain indirect fitness benefits by increasing the number of sexuals that the colony produces, their defensive role through phragmosis could not be confirmed (Roux and Korb 2004). (iv) Additionally, after collecting more than 600 colonies over 5 years we never found predators or signs of predation (e.g., unusually shaped and/or unsealed openings). The ant species present (*Camponotus*, *Polyrhachis*, *Monomorium*) were indifferent to the termites and vice versa (J. Korb and E. A. Roux unpublished observations). The only potential threat for a colony resided in putative competitors such as wood feeding beetle larvae (Buprestidae, Cerambycidae) and termite colonies, either conspecific or congeneric (*Cryptotermes domesticus*), which together occurred in more than 50% of all trees inhabited by *C. secundus*. However, blocking of galleries

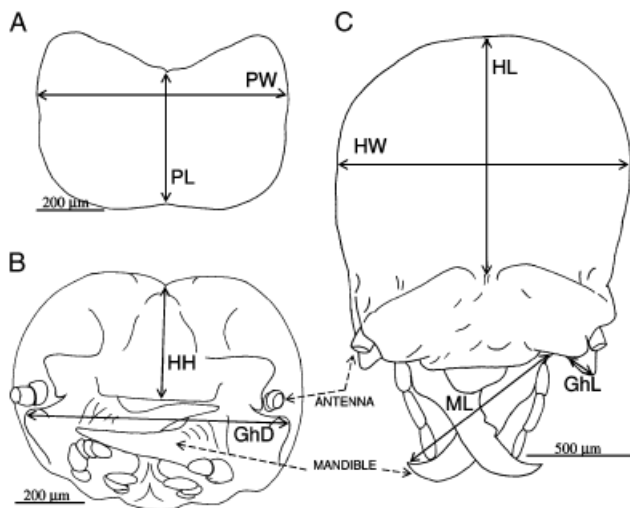
appears to be an inefficient defense against such wood-dwelling competitors which are effective tunnellers. As it is difficult to observe and infer the fitness consequences of rare (predation) events directly we used morphological traits as indicators for selection. We compared the morphological evolution of traits involved in defense (i.e., biting and plugging) with that of nondefensive traits. A negative allometric relationship of defensive traits to body size would indicate that phragmotic defense is under stabilizing selection, that is that it is still functional (e.g., Eberhard et al. 1998, 2000; Emlen and Nijhout 2000; Ohno et al. 2003). This assumes ongoing stabilizing selection with a fairly rapid mutation rate, so that a mutation-selection balance is continually maintained. Knowledge of the genetic basis of these traits is lacking. However, phylogenetic data for this family suggest that phragmotic traits are under continuous selection, evolving rapidly, but also being lost twice independently (Thompson et al. 2000). Similar implications were revealed for another termite with phragmotic defense, *Reticulitermes speratus* (Rhinotermitidae) (Matsuura 2002).

The second aim of our study was to compare the scaling relationships of defensive traits in our sterile soldiers with those from weapons in other species in which the individuals face a trade-off between investment in defense and reproduction (e.g., Emlen and Nijhout 2000; Simmons and Emlen 2006). In the latter, weapons are generally exaggerated male traits which are selected by intra- and intersexual selection (reviewed by Emlen and Nijhout 2000). Comparing the scaling relationships between both “types” of weapons will provide insights into how different evolutionary forces (natural selection in termite soldiers vs. sexual selection in nonsocial insects) and constraints (specialization on defense in sterile termite soldiers vs. trade-off between “weapons and testes” in nonsocial insects) shape trait evolution.

## METHODS

### Insects and morphological measurements

Colonies of *C. secundus* were collected in the mangroves of Darwin Harbor (Northern Territory, Australia). Soldiers ( $N = 161$ ) found during the census of 44 colonies were preserved in ethanol (90%). Eight traits (Fig. 1) from the head and the pronotum were measured with an eyepiece micrometer on a dissecting microscope ( $\times 800$ ) with recordings to the nearest 0.01 ml, and categorized as defensive and nondefensive. The defensive category comprised maximum head width (HW), maximum head height (HH), maximum distance between the genal horns (GhD), and maximum mandible length (ML) (Fig. 1). The nondefensive traits were maximum pronotum width (PW), minimum pronotum length (PL), maximum genal horn length (GhL), and maximum head length (HL) (Fig. 1). When a complete antenna was present, the antennal segments were counted as they indicate the instar from which the soldier differentiated (Roisin 2000). Additionally, when the



**Fig. 1.** (A) Dorsal view of soldier pronotum, PW, pronotum width; PL, pronotum length. (B) Frontal view of soldier head, HH, head height; GhD, genal horn distance. (C) Dorsal view of soldier head, HW, head width; HL, head length; GhL, genal horn length. Both antennae were cut and only the first two segments are shown.

abdomen was preserved well enough, sex was determined using external abdominal characters (Roonwal 1975; Grassé 1982b). To check for a possible match between soldier heads and the galleries of their nest, the minimum and maximum diameters of 115 galleries of 16 colonies (five or 10 galleries per colonies upon availability) were measured (respectively minG and maxG) with a caliper.

If there is stabilizing selection on soldier heads, it might be general selection acting on all members of a termite colony. To test this hypothesis we used 10 alates from each of nine colonies as control measurements. We measured the minimum head width between the eyes (Hwi), the maximum head length (HL), plus PW and PL as measured in soldiers. Alate sex ratio does not differ from equity (J. Korb unpublished data), and both sexes were equally represented in our sample.

## Statistics

### Biometric analysis

We used the principal component 1 (PC 1) as an overall measure for body size rather than a single trait as size indicator. The PC 1 scores were calculated from the variance-covariance matrix of the raw measurements of the eight body traits, all measurements being in the same unit. To calculate the PC 1 scores, the constant was set at zero to prevent negative PC 1 values. This allowed  $\log_{10}$ -transformation of the scores. For each trait, the allometric slope was estimated by linear regression analysis (ordinary least square, OLS) of the  $\log_{10}$ -transformed value of the trait on the  $\log_{10}$ -transformed PC 1 score. Such an estimation of slopes is unaffected by differences in the size of measurement among traits (Smith 1980). The estimates of the regression slopes were tested against the null hypothesis of isometry (slope = 1) with *t*-tests. Such regression slopes are presumably the result of selection favoring one slope over another, and thus reflect the design features of an organism (Eberhard et al. 1998, 2000). Another important aspect of variation in the size

of a trait is the relative amount of variation of that specific trait, compared with that of other traits. This is usually expressed as the coefficient of variation (CV, i.e., the standard deviation divided by the mean). However, Eberhard et al. (1998) pointed out that the CV is affected by the regression slope and by the degree of dispersion around the line of that slope. The latter is related to internal and external factors affecting an individual's development as well as "developmental noise" (i.e., random variations in the expression of a developmental program). To assess solely the degree of dispersion, we used  $CV'$  which is the CV on the residuals of body size.  $CV'$  is expressed as  $CV \times (1 - r^2)^{1/2}$  where *r* is the correlation coefficient of the trait with body size (see Eberhard et al. 1998; Ohno et al. 2003). The allometric slope, CV and  $CV'$  were compared between defensive and nondefensive traits with Mann–Whitney *U*-tests.

### Sources of variation

The effect of colony on each morphological trait and on the two gallery diameters was analyzed with analysis of variance (ANOVA). For the morphological traits, we used only colonies with a minimum of four soldiers as we needed a good estimate of the variance within a colony. The homogeneity of variances was tested with Levene tests (Levene 1960; Brown and Forsythe 1974) and for all but three variables (GhD, HL, and minG) the colonies' variances were not homogenous (Levene test, HW:  $F_{16,98} = 1.97$ ,  $P < 0.05$ ; HH:  $F_{16,98} = 1.75$ ,  $P < 0.05$ ; PW:  $F_{16,98} = 1.82$ ,  $P < 0.05$ ; PL:  $F_{16,98} = 13.77$ ,  $P < 0.001$ ; GhL:  $F_{16,98} = 3.94$ ,  $P < 0.001$ ; ML:  $F_{16,98} = 1.95$ ,  $P < 0.05$ ; maxG:  $F_{15,99} = 1.811$ ,  $P < 0.05$ ). Thus, for these variables, the usual *F* test could not be applied. However, the usual *F* ratios can be tested by a Monte-Carlo procedure (Manly 1997). Let  $F_0$  be the ratio to be assessed. A set of *N* simulated data sets is obtained by permuting the labels of the colonies. For each permutation the *F* statistic is recomputed and compared with the real value  $F_0$ . Let *K* be the number of times where  $F > F_0$ ; then the proportion  $P = K/N$  represents the probability that a random data set, similar to the real one, provides an *F* equal to or larger than the observed  $F_0$ . It is compared with the usual 5% threshold. If *P* is  $< 5\%$  then the test is said to be significant, that is to say there is a difference between the mean values associated to the factor under study. In fact, in agreement with the null hypothesis, the real non-permuted data set may be joined to the permuted sets, so that only *N* – 1 permutations are performed. In the present study the number *N* was set equal to 10,000. The procedure was rewritten according to Manly (1997, Chapter 5, appendix A): FORTRAN subroutines for one, two, and three factors ANOVA.

The relationship between the gallery diameters of each colony and its soldiers size was estimated by two linear regression analyses (OLS) of the  $\log_{10}$ -transformed mean diameter per colony on (i) the  $\log_{10}$ -transformed mean PC1 scores and (ii) the  $\log_{10}$ -transformed mean HW of these colonies. The effects of "age" (i.e., instar at soldier differentiation) and sex on each morphological trait were analyzed using ANCOVA with "age" as the covariate.

The slopes, CV and  $CV'$  of alate traits, the control measurements, were compared with those of soldier defensive traits with Mann–Whitney *U*-tests. The effect of sex on alate traits was tested with ANOVA.

## RESULTS

### Biometric analysis

#### Soldiers

The first principal component (PC1) explained 55.31% of the total variance in soldiers, and all the eight traits had a positive loading on PC1. PC1 thus represented the overall body size. The coordinates on this axis can be explicitly expressed as a linear combination of the morphological variables:  $PC1 = 0.414 HW + 0.431 HL + 0.138 HH + 0.491 PW + 0.545 PL + 0.261 GhD + 0.050 GhL + 0.121 ML$ .

The allometric slopes for the nondefensive traits were either  $>1.0$  (PL) or they did not significantly deviate from 1.0 (PW, HL, GhL; Table 1, Fig. 2). In contrast, all slopes for the defensive traits were significantly  $<1.0$  (HW, HH, GhD, ML; Table 1, Fig. 3). The slopes for defensive traits were significantly less than the slopes for nondefensive traits (Mann–Whitney *U*-test,  $Z = -2.021$ ,  $P < 0.05$ ).

Defensive traits had lower CV than nondefensive traits (Table 1), and this difference was significant (Mann–Whitney *U*-test,  $Z = -2.021$ ,  $P < 0.05$ ). However, the strict measure of dispersion around the allometric line,  $CV'$ , did not differ significantly between the defensive and nondefensive traits (Mann–Whitney *U*-test,  $Z = -1.732$ , NS).

#### Alate controls

The first principal component on alate traits (PC1a) explained 50.94% of the total variance, and the four traits had positive loadings on PC1a. PC1a represented alate body size with  $PC1a = 0.22 HL + 0.84 PW + 0.26 PL + 0.42 Hwi$ . The allo-

metric slopes of alates PL and HL were  $<1.0$ , whereas the PW slope was larger and the slope of HW did not deviate from 1.0 (Table 1). The slopes, CV and  $CV'$  of alate traits did not differ significantly from those of the soldier defensive traits (Mann–Whitney *U*-test,  $Z = -0.866$ ;  $Z = -0.577$ ;  $Z = -0.289$ , respectively, all NS).

### Sources of variation

#### Sex and “age”

The 128 soldiers for which sex was determined consisted of 105 (82%) females and 23 (18%) males. This sex ratio differed significantly from a 1:1 ratio ( $N = 128$ ,  $\chi_1^2 = 52.5$ ,  $P < 0.001$ ).

ANCOVAs indicated that for all traits the regression slopes did not differ significantly between male and female soldiers (Table 2). Moreover, for three traits (HW, GhD, and HL) these regression lines correlated with the number of antennal segments, that is these traits were smaller for soldiers that differentiated at an earlier instar. These traits (HW, GhD, HL) showed significant sex differences in the intercepts of the regression lines (ANCOVAs Table 2, HW in Fig. 4), that is males that differentiated at a young instar were smaller for these traits than their female counterparts. In alates, none of the four traits measured differed significantly between males and females (ANOVA, Hwi:  $F_{1,88} = 0.185$ , NS; HL:  $F_{1,88} = 0.079$ , NS; PW:  $F_{1,88} = 0.051$ , NS; PL:  $F_{1,88} = 0.415$ , NS).

#### Colony

There was a significant difference between colonies for all measured soldier traits (ANOVA, Table 3). Similarly, the

**Table 1. Allometric slopes and other features of phenotypic variation for eight morphometric traits of *Cryptotermes secundus* soldiers ( $N = 161$ ) and for four traits of alates ( $N = 90$ )**

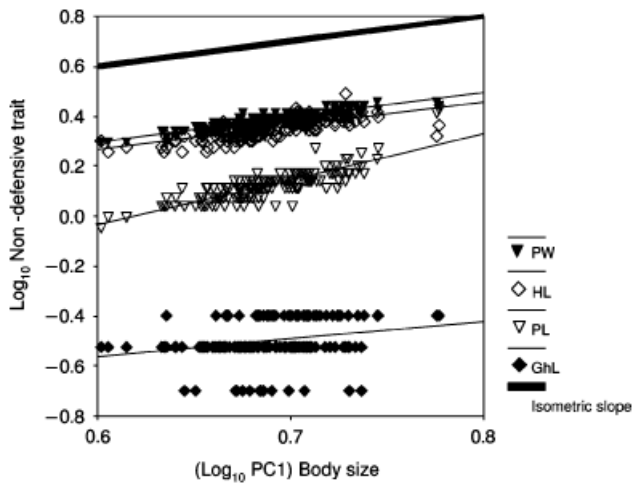
Trait category	Morphometric traits	Mean (mm)	slope	CV	<i>r</i>	$CV'$
Defensive	Head width	1.29	0.80*	0.07	0.80*	0.04
	Head height	0.58	0.58*	0.09	0.40*	0.09
	Genal horn distance	1.09	0.59*	0.05	0.72*	0.04
	Mandible length	0.74	0.42*	0.07	0.40*	0.04
Nondefensive	Pronotum width	1.21	0.99 NS	0.08	0.85*	0.04
	Pronotum length	0.68	1.80*	0.16	0.84*	0.09
	Head length	1.13	0.96 NS	0.07	0.72*	0.46
	Genal horn length	0.16	0.71 NS	0.18	0.25*	0.18
Alate traits	Head width	0.80	0.91 NS	0.07	0.63*	0.06
	Pronotum width	1.05	1.35*	0.09	0.93*	0.03
	Pronotum length	0.67	0.64*	0.08	0.48*	0.07
	Head length	1.21	0.28*	0.06	0.31*	0.06

As an indicator of body size, the principal component 1 score based on the variance covariance matrix of the eight traits was used. Significance tests for regression slopes and correlation coefficients were carried out against the null hypothesis of slope = 1 and of  $r = 0$ , respectively.

\*  $P < 0.05$ .

Slope: slope of regression line of a trait on body size (both variables  $\log_{10}$ -transformed). CV: coefficients of variation (standard deviation divided by the mean) calculated for nontransformed variables. *r*: Pearson's product-moment correlation coefficient between a trait and body size.  $CV'$ : a measure of the degree of dispersion of data points around the regression line, expressed as  $CV \times (1 - r^2)^{0.5}$ .

NS, nonsignificant.

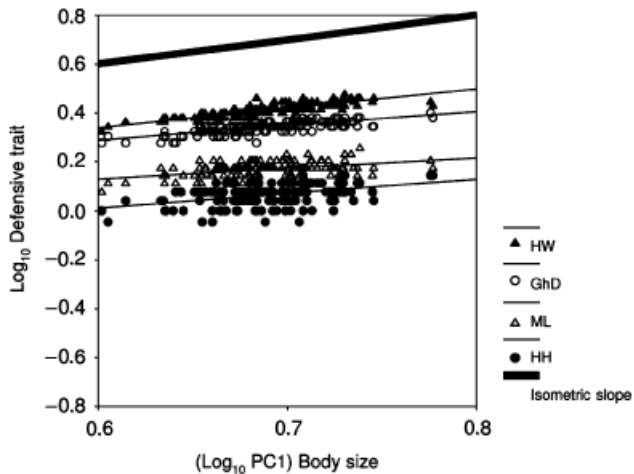


**Fig. 2.** Comparison of the allometric slope of each nondefensive trait with an isometric slope (bold line). Only the slope for PL was >1.0, the other three slopes did not significantly deviate from 1.0 (Table 1). Abbreviations as in Fig. 1.

minimal gallery diameter differed significantly between colonies, whereas there was no significant difference for the maximal diameter (ANOVA, Table 4).

**Match with galleries**

The gallery diameters' CV were of the same order of magnitude as the soldiers' defensive traits (minG, CV = 0.06; maxG, CV = 0.05). The correlations between gallery diameters, for maximal and minimal distance, and soldier body size were not significant (maxG,  $F_{1,14} = 2.73$ , NS; minG,  $F_{1,14} = 3.69$ , NS) whereas the correlations with head width



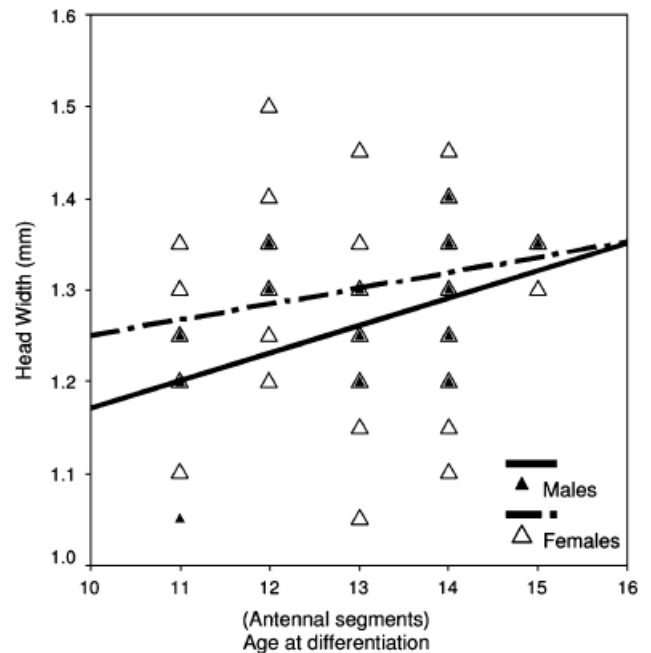
**Fig. 3.** Comparison of the allometric slope of each defensive trait with an isometric slope (bold line). All slopes were significantly <1.0 (Table 1). Abbreviations as in Fig. 1.

**Table 2.** ANCOVAs on head width (HW), head length (HL), head height (HH), pronotum width (PW), and pronotum length (PL) between the sexes with the number of antennal segments (Antennalseg.) as covariate (88 females, 17 males)

Fixed factor [df]	Antennalseg. [1]		Sex [1]		Sex × antennalseg. [2]	
	F	P	F	P	F	P
HW	8.29	<0.01	3.31	0.07	5.22	<0.01
HH	0.246	NS	0.016	NS	0.136	NS
GhD	9.49	<0.01	0.26	NS	4.73	<0.05
ML	0.064	NS	0.019	NS	0.042	NS
HL	9.52	<0.01	0.29	NS	4.81	<0.01
PW	3.82	0.053	0.32	NS	1.99	NS
PL	2.75	NS	0.70	NS	1.59	NS
GhL	3.01	0.08	0.04	NS	1.53	NS

Sexes did not differ significantly; however, males that differentiated at a younger instar had smaller HW, GhD and HL than “young” females. NS: nonsignificant, exact P values are given between 0.1 and 0.05.

were both significantly different from zero (maxG,  $F_{1,14} = 9.99$ ,  $P < 0.01$ ; minG,  $F_{1,14} = 6.54$ ,  $P < 0.05$ ) with a coefficient of determination of 42% and 32%, respectively. Colonies with soldiers that had large head widths had larger gallery diameters.



**Fig. 4.** Males and females head width were not significantly different, but “older” individuals had larger heads. The interaction between both factors was significant: young males had smaller HW than young females (Table 4).

**Table 3. ANOVAs on head width (HW), head height (HH), genal horns distance (GhD), mandible length (ML), head length (HL), pronotum width (PW), pronotum length (PL), and genal horn length (GhL) among colonies (115 soldiers, 17 colonies, all with a minimum of four soldiers)**

Trait category	Variables	BSS	WSS	F	P
Defense	HW	1.16	1.47	4.82	<0.001
	HH	0.40	1.14	2.16	<0.05
	GhD	0.59	1.01	3.54	<0.001
	ML	0.29	0.60	2.92	<0.001
Nondefense	HL	1.15	3.60	1.96	<0.05
	PW	1.41	2.54	3.40	<0.001
	PL	3.14	3.22	5.97	<0.001
	GhL	0.13	0.28	2.88	<0.001
df		16	98		

All variables differed between colonies.  
BSS, between sum of square; WSS, within sum of square.

## DISCUSSION

We investigated the scaling relationship between size, shape, and defensive functions in the phragmotic termite soldier, *C. secundus*, to test whether plugging is selectively maintained. The allometric values and CVs of defensive traits were significantly less than those of nondefensive traits, whereas the strict measure of dispersion, CV', did not differ between the two categories. These results show (i) that the size of soldier's defensive traits are more stable against a change in body size than nondefensive traits, and (ii) that the difference in degree of phenotypic variation is accounted for by the different changes relative to body size (i.e., difference in design feature). This is the first indication that defensive traits of *Cryptotermes* soldiers are under stabilizing selection and the negative allometry of this hemimetabolous insect presents a new case of insect developmental allometry which is not explained by sexual selection, as argued for holometabolous insects (Eberhard et al. 1998; Stern and Nijhout 1999; Emlen and Nijhout

2000; Ohno et al. 2003). Alternatively, the negative allometry could be interpreted as evidence for developmental or genetic trade-offs, for instance due to pleiotropic effects. However, we think that is unlikely because then we would expect the same scaling relationships for the same traits in other castes. Indeed, we found no differences in allometric values nor in CV or CV' between soldier defensive traits and alate traits. All alate traits, except Hwi, appear to be stabilized. At first this seems to indicate that stabilization on the defensive traits of soldiers is not restricted to this caste. However, all alate traits behaved differently to changes in body size compared with soldier traits (e.g., HW had a negative allometry in soldiers, but was isometric in alates; PL had a positive allometry in soldiers and a negative in alates). Thus, although selection is acting on alate morphology, the stabilizing selection acting upon the defensive traits of soldiers is very likely to be specific to this caste.

The present findings are congruent with previous untested statements on the phragmotic function of *Cryptotermes* soldiers (Deligne et al. 1981; Grassé 1982a; Prestwich 1984). Thus, although predation pressure is low in *C. secundus* and plugging traits were lost in a closely related species (*Procrystotermes australiensis*; Thompson et al. 2000), phragmotic defense is maintained in *C. secundus* soldiers and may be the unique behavior specific to this caste while other behaviors (e.g., biting and alarming) do not differ (Roux and Korb 2002). Until now stabilizing selection has been studied only in one rhinotermitid (Matsuura 2002). Here, HW was also found to be less variable than other body parts, however sexual dimorphism was more pronounced (see also Matsuura 2006). The latter might be due to the more "derived" state of the Rhinotermitidae compared with the Kalotermitidae (Roisin 2000) and anticipates the sex-specific caste patterns observed in some higher termites where one sex is strictly associated with one caste (Roisin 2000).

On a proximate level, the adjustment of soldiers' HW around the colonies' gallery dimensions can be simply achieved if one assumes that smaller false worker instars hollow smaller galleries than larger instars. In incipient colonies the majority of false workers are small and soldiers develop from small instars, whereas in mature colonies the worker force is composed of various sized instars and soldiers develop from "older," that is, larger, instars (Noirot 1985; Crosland et al. 2006). Thus when the galleries get larger as the false worker instars grow the new developing soldiers are also larger. This is consistent with our results showing that even stabilized traits are colony specific. Compared with males, female soldiers were larger when they developed from a small instar, thus they might be more effective at plugging and the female biased sex ratio might be adaptive in incipient colonies. However, both the size dimorphism of early instars and the female bias observed in late instars remain unexplained. New data suggest that behavioral differences between both

**Table 4. ANOVAs on the minimum and maximum diameter of galleries (N = 115) among 16 colonies**

Variables	BSS	WSS	F	P
minG	0.87	2.48	2.31	<0.01
maxG	0.51	2.34	1.43	NS
df	15	99		

BSS and WSS as in table 2; NS, nonsignificant; ANOVA, analysis of variance.

sexes might explain the female biased soldier sex ratio in *C. secundus* (Muller and Korb 2008).

Our results for sterile soldiers are in striking contrast to what is known for defensive traits/weapons in nonsocial insects. Here, weapons are generally exaggerated male traits that increase disproportionately with overall body size (Emlen and Nijhout 2000). This difference could be due to fact that *C. secundus* soldiers were mainly females. But, although less applicable to solitary insects, we would expect a similar trade-off between the development of ovaries and weapons as between the development of testes and weapons. There are, however, at least two major evolutionary differences between the weapons of termite soldiers and males from nonsocial insects. First, the termite soldiers are sterile and have an exclusively defensive function. By contrast, the weapons of other insects generally serve other purposes. They are mainly traits evolved by sexual selection, either due to female choice or male–male competition over access to female mating partners (Simmons and Emlen 2006), and it has been suggested that the scaling component reflects the relative fitness advantages of exaggerated traits versus somatic growth (Kodric-Brown et al. 2006). Thus, despite the common term “weapon” the selective forces shaping the evolution of these traits differ fundamentally and exaggerated traits are selected in males of solitary insects, while a disproportional over-scaling is not necessarily favored by natural selection through predators. Although certainly more data are needed to reveal a broader pattern, this study shows that one weapon is not like another—an aspect that has been largely neglected so far. Second, recently it has been shown convincingly that there seems to be an evolutionary trade-off between weapons and fecundity traits (Simmons and Emlen 2006). In termite soldiers this trade-off does not exist as they are always sterile. So individual specialization on defense is not constrained by fecundity losses. However, the trade-off between defense and fecundity might be reflected in the caste composition of termite colonies; the caste for defense, the soldiers, are always sterile and the reproductives generally lack defensive adaptations. A notable exception are “neotenic soldiers” (i.e., replacement reproductives that have soldier like defensive traits) which are restricted to the Termopsidae (Roisin 2000; Thorne et al. 2003). It would be interesting to test whether there is a trade-off between fecundity and defensive traits in neotenic soldiers similar to that found in solitary insects. The third termite caste, the workers, have a variable position with regard to fecundity, depending on the species, its life type, and its degree of task specialization (Shellman-Reeve 1997). The termite’s caste pattern might imply that some evolutionary trade-offs that constrain the optimal development of individuals in solitary species have been uncoupled in termites through the evolution of different castes. It might be promising to ask, from an evolutionary developmental point of view, whether division of labor and caste differentiation in social insects generally

means an uncoupling of developmental constraints. When individuals in social insect are “released” from developmental constraints through the evolution of castes, this certainly contributed to the ecological and evolutionary success of social insects.

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