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Polymorphism of female reproductives in the tramp ant *Technomyrmex vitiensis* (Hymenoptera: Formicidae: Dolichoderinae)

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Abstract

Colony reproduction in the tramp ant *Technomyrmex vitiensis* MANN, 1921 is characterized by the co-occurrence of dispersing winged queens and local, wingless, intermorphic reproductives, similar to other species of the *T. albipes* SMITH, 1861 group. A morphometric analysis of intermorphs and workers separated the two castes and developmental character of ocelli and size of compound eye clearly correlate with ovarian development. Intermorphs constitute a substantial proportion of the colony, but in our sample only a small fraction of intermorphs were inseminated despite the presences of winged males. Wingless males, which mate with intermorphs in other *Technomyrmex* species, have as yet not been found in *T. vitiensis.* Our data support earlier findings from closely related *Technomyrmex* that reproduction is based on an opportunistic strategy enabling the species to rapidly colonize new habitat. An outbreak of a colony fragment into the walls of our laboratory was efficiently stopped by use of boric acid baits, indicating that trophallaxis is used to distribute food.

Key words: *Technomyrmex vitiensis*, intermorphic queens, caste system.

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Introduction

The evolutionary and ecological success of ants is to a large extent founded on a pronounced female polymorphism, with queens and one or several types of workers functionally and morphologically specialized for their specific tasks. In addition, female reproductives of many ant species exhibit a similarly striking polymorphism tightly linked to alternative reproductive and dispersal tactics. In many species, founding queens disperse on the wing and start a new colony solitarily, using their internal fat reserves and digested flight muscles to rear their first offspring (HÖLLDOBLER & WILSON 1990). Monogynous species with independently founding queens are usually characterized by a long reproductive period with high queen fecundity, but the queens' death usually leads to the decline of the colony. Independent founding is associated with increased gene flow and reduced sibling competition, but an individual queen's probability of surviving the initial phase of colony foundation is extremely low (HÖLLDOBLER & WILSON 1990, JOHNSON 2006). In contrast, colonies of species with dependently founding queens may recruit their own female sexuals to guarantee colony survival beyond the longevity of a single queen. Dependently founding queens are often characterized by a short reproductive period and low fecundity but a high probability of surviving until maturity (HÖLLDOBLER & WILSON 1990). They are often smaller in size and pose lower investment costs to the colony than independent queens, which are provisioned with large body resources. Whenever dispersal abilities are limited and mating occurs among nestmates, strategies must have evolved to minimize the constraints resulting from inbreeding and intercolonial competition.

In some cases, dependently founding queens have lost their wings and other adaptations for flight and may resemble the non-reproductive worker castes in their external morphology and size (HEINZE 2008). Such queens are thus almost as cheap and fast to produce as workers. As numerous terms have been proposed for wingless reproductives, we here adopt the function-based terminology proposed by BUSCHINGER & WINTER (1976) and refer to them as "intermorphic queens" (further abbreviated with "IM") to distinguish them from irregularly occurring, pathological intermediates without special function (HEINZE 1998).

In this study we focus on the genus *Technomyrmex*, which is remarkable in two ways.

First, it contains many widely distributed, opportunistic tramp species, with *T. albipes* (SMITH, 1861) (and other "white-footed ants", namely T. *vitiensis*, *T. difficilis* FOREL, 1892 and *T. pallipes* (SMITH, 1876) see BOLTON 2007) probably the most prominent. *Technomyrmex* is therefore of considerable interest to conservation biologists (e.g., WARNER & SCHEFFRAHN 2004, 2005). Second, a large number of species within *Technomyrmex* have evolved a mode of colony reproduction that combines dispersal by winged queens and colony propagation by wingless, intermorphic replacement reproductives. Individuals, which are morphologically intermediate between queens and workers, are presently known from 25 of the 43 described species of the *T. albipes* group (BOLTON 2007). They lack the pronounced mesonotum associated with the wing apparatus of female reproductives that disperse by flight and thus superficially resemble normal workers.

The reproductive biology of intermorphs has been described in detail for *T. brunneus* from Japan (referred to as *T. albipes*, TSUJI & al. 1991, YAMAUCHI & al. 1991, OGATA & al. 1996). Winged males and females are produced once a year and disperse synchronously in summer. After mating, winged queens found new colonies independently. Once the colonies grow, IMs are produced, which mate in the nest with wingless males and supplement and finally replace the queen. About half of all individuals in larger colonies may be inseminated IMs. Such colonies grow quickly and spread through fission, i.e., groups of IMs and workers leave the nest and initiate new colonies nearby. *Technomyrmex pallipes* (see BOLTON 2007) and *T. difficilis* appear to show a similar life history (DEYRUP 1991, WARNER 2003). Here we provide more detailed data on the caste system and life history of still another species, *T. vitiensis* MANN, 1921. More precisely, because of superficial morphological overlap of IMs and workers, we describe unique characters for reproductive and non-reproductive castes by analyzing internal and external characters.

Materials and methods

Collection

Technomyrmex vitiensis is a species presumably of Southeast Asian origin, which has been introduced into European and US greenhouses of botanical gardens where it forms large supercolonies. Like other species of the *Technomyrmex albipes*-group, *T. vitiensis* is a tramp ant species, and is widely distributed throughout greenhouses and botanical gardens, though it is unclear whether it negatively affects the plants (e.g., BOLTON 2007) or on the contrary preys on herbivores and plant parasites (GAUME & al. 2005). In May 2005 we collected *T. vitiensis* in the botanical garden of Bonn University, Germany. It inhabits the warm house in large numbers, being an annoying pest to the gardeners. Like *T. brunneus* (see TSUJI & al. 1991), *T. vitiensis* forms polydomous nests. The ants nested opportunistically in cavities in plants and did not construct special nest structures. Removal of the outer layers of palm trees almost always revealed workers, intermorphs, and winged males together with brood. Groups of workers, intermorphs, and brood were also found on the inner side of large leaves and other highly instable nest sites, either indicating that optimal nest sites have become limited in the greenhouse or that predation pressure is low. Despite intensive search we could not find winged or dealate female sexuals or wingless males.

Housing conditions and pest control

We transferred colony fragments into 20 cm × 20 cm × 10 cm plastic boxes with plaster flooring and small cavities in the plaster provided as nest sites. We maintained the ants for 3 months at a 12 - 12h light-dark regime at 26°C and 60% humidity and fed chopped cockroaches and diluted honey twice a week.

Though the walls of the plastic boxes were regularly coated with Fluon®, some ants escaped from one box and for a few months inhabited hidden cavities in the walls of the climate chamber or the tubing system. For a few weeks, foragers from this nest were occasionally seen at night and during the lesser frequented weekends also during the day, and groups of workers were sometimes found at water bottles and other moist areas on the work benches. Here, we collected three winged females together with workers, but neither brood nor males were present. The queens were stored in ethanol for later analysis. The outbreak was eventually stopped using filter papers soaked with a sugar solution containing 2% boric acid (suggested by A. Suarez; see also KLOTZ & MOSS 1996, KLOTZ & al. 1997, 1998, 2000). Boric acid is less toxic than other commercially available pest control agents. It affects the nervous system and, because it acts slowly, is readily spread by trophallaxis throughout the whole colony. Crucial for the success of this treatment is that food is exchanged among individuals by trophallaxis. The exchange of liquids among adults has never been observed in *T. brunneus* and instead trophic eggs appear to play a crucial role in nutrition (YAMAUCHI & al. 1991). In contrast, we frequently observed trophallaxis in *T. vitiensis*, allowing for the toxin to spread throughout the colony. We renewed filter paper baits every evening over a period of two weeks. We regularly found dead ants, but not necessarily in the vicinity of the bait. After two weeks of applying baits we observed no new *T. vitiensis*.

Morphometry

For the morphological analysis of IMs and workers we chose 68 ants roughly covering the whole range from small to large individuals. To assess whether morphometric characters reliably discriminate workers from IMs, we measured five continuous characters under a binocular to the nearest 0.01 mm at 20× magnification using pinned specimens:

- HW Head width; maximum width of cephalic capsule, measured in full-face view at posterior margin of eyes.
- HL Head length; midline length of cephalic capsule in full-face view from anterior margin of clypeus to midpoint of posterior margin of occiput.
- SL Scape length; maximum (diagonal) length of a single scape per specimen, taken in anterior view.
- PW Pronotum width; maximum width of pronotum, taken in dorsal view.
- ML Mesosoma length; maximum length measured from anterior margin of pronotum to posterior margin of propodeum, taken in lateral view.

In addition we investigated three characters generally thought to be associated with queen anatomy (presence/absence of ocelli; presence/absence of spermatheca, number of ovarioles). When the typical iridescence of the spermatheca indicated the presence of sperm, the spermatheca was also studied at higher magnification under a binocular microscope.

Because continuous morphometric external variables deviated from normality data were log-transformed. To overcome conflicts associated with colinearity of morphometric data we reduced the number of characters by a principal component analysis (eigenvalues > 0.80). We compared the extracted factors using a t-test with "caste" as grouping variable based on the presence or absence of ocelli ("worker", n = 46; "IM", n = 22). Missing data was substituted by the corresponding mean.

To test whether external morphology was a good predictor of caste we performed a discriminant analysis based on the extracted factors (see above) using Statistica 13.0. Again, individuals were *a priori* grouped as "worker" or "IM" based on the presence or absence of ocelli and the characters were entered together.

To further discriminate workers and IMs from winged queens we counted the ommatidia of one compound eye each of three winged queens, eight IMs, and eight workers using scanning electrone microscope visualization using a magnification of 160 - 400×. Because the number of queens was limited we do not have data from their ovaries.

Results

Morphometry

Our study revealed a clear association between ovarian anatomy and ocelli. Ocelli (appearance ranged from traces to a full set of three ocelli) were present in 22 of 68 examined individuals (32%), all of which had ovaries consisting of 10 to 24 ovarioles (median 16.5; quartiles 15 and 19.75, Fig. 1f) and a spermatheca. Individuals without ocelli did not have a spermatheca and always four ovarioles.

Except for the number of ommatides the external morphology revealed considerable overlap across caste (Fig. 1a - e) and none of the characters clearly indicated whether an individual is reproductive. Again, eye development (Fig. 1g) is a reliable predictor of caste. Number of ommatidia (mean ± SE) differed significantly between winged queens (107.00 ± 1.42), IMs (70.13 ± 1.19) and workers (54.76 ± 1.42) (one-way ANOVA, Fishers' post hoc test; df = 16; $p < 0.001$).

The factor analysis reduced the five external morphological characters to two factors (PC1 and PC2) that combined explained 78.4% of the variance (PC1 = 60.7%; PC2 = 17.7%). The single contributions (factor loadings) of the characters cumulated to the PC1 by HW (-0.78) + HL (-0.87) + SL (-0.53) + PW (-0.80) + ML (-0.87) and to PC2 by HW (0.03) + HL (-0.01) + SL (0.82) + PW (-0.46) + ML (-0.05). The low factor loading of SL in PC1 together with its positive contribution to PC2 is caused by an opposite trend, as all other characters were larger in intermorphic queens. Despite the above described overlap of single morphometric characters, IMs therefore are distinct from workers in external morphology when combining the morphometric data (t-test, df = 66; PC1 (t = -6.21), $p < 0.001$; PC2 (t = -6.56), $p < 0.001$).

In the discriminant analysis based on PC1 and PC2 all but three individuals were correctly classified to groups *a priori* defined based on the absence or presence of ocelli (one "worker" without ocelli and with 4 ovarioles was classified as IM; two IMs with ocelli and 16, respectively 17 ovarioles were classified as workers, Fig. 2). It is noteworthy that the latter IM was one of the two inseminated specimens. Nevertheless, IMs and workers differed significantly in continuous morphological traits (Wilks-Lambda 0.24, F = 104.95, $p < 0.001$) in addition to ovarian anatomy and the development of ocelli.

A closer examination of the ovaries of IMs revealed the presence of sperm in one spermatheca (Fig. 3) and traces of sperm in a second.

Discussion

Like other species of the *Technomyrmex albipes*-group, *T. vitiensis* shows two distinct types of female reproductives: winged queens, which are apparently produced only under certain environmental conditions and presumably disperse and colonize new habitat patches, and wingless replacement reproductives, which mate in the nest and locally increase colony size. Intermorphic reproductives can reliably be distinguished from workers by the presence of ocelli, number of ommatides and ovarian anatomy. They are also distinct in external morphology, although both castes show some degree of size variation in all measured external characters. Similar to *T. brunneus* and *T. difficilis,* our sample of *T. vitiensis* indicates that IMs constitute a considerable proportion (32%) of the colony. So far no study has addressed whether specific mechanisms such as hierarchies regulate the respective contributions of the reproductive individuals to the brood. *Technomyrmex* represents an inter-

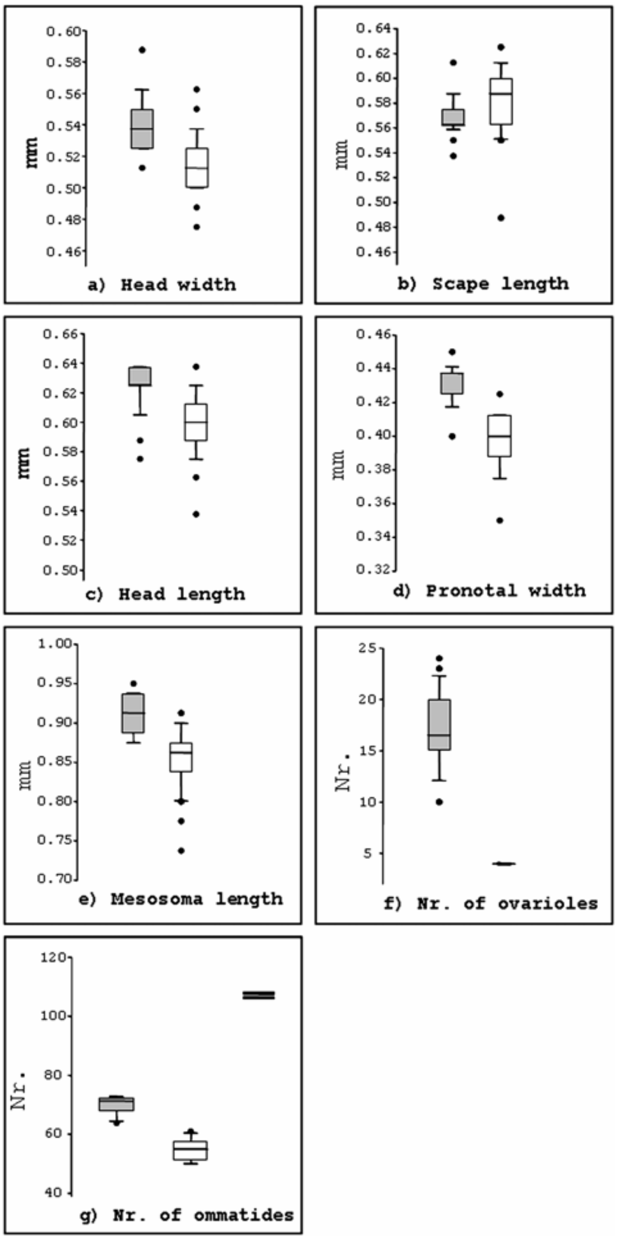

Fig. 1: Boxplots of the characters used for the analysis (a - g). Different colour indicates caste assessed by dissecting the ovaries: Grey = intermorph (n = 22), white = worker (n = 46), black = winged queen (n = 3, Fig. 1g). Black filled circles depict datapoints that lie outside the 10th and 90th percentiles.

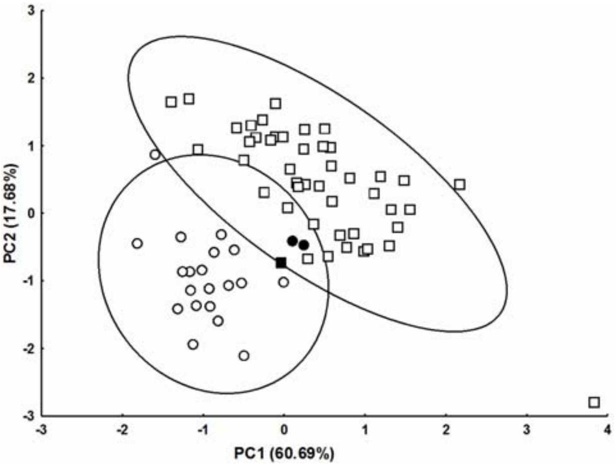

Fig. 2: Discriminant function of intermorphs (circles) and workers (squares) based on two principle components. Prediction interval ellipses for each caste are based on 95% probabilities. Closed symbols indicate individuals that were incorrectly classified in the DA.

Fig. 3: Spermatheca of an inseminated intermorph of the ant *Technomyrmex vitiensis*. Only few spermatozoa were present (see text). Picture taken at 100× with a ZEISS Axiophot microscope.

esting case with a predicted high degree of cooperation among sexually active individuals as a result of secondary polygyny (YAMAUCHI & al. 1991).

In spite of the presence of numerous winged males, the spermatheca was full in only one IM and partly filled in another, and we also could not observe mating attempts. In *T. brunneus*, IMs usually mate with wingless, ergatoid males (YAMAUCHI & al. 1991) and the copulatory organs of winged and wingless individuals differ considerably in size (OGATA & al. 1996). Ergatoid males have been found in six species of the *T. albipes* group. One possible explanation for the low number of inseminated IMs might therefore be that winged males of *T. vitiensis* are similarly specialized for mating exclusively with winged queens, while IMs mate with ergatoid males. However, we neither found winged queens in the greenhouse nor ergatoid males under any condition, although we cannot rule out that we missed ergatoid males. IMs might therefore occasionally be capable of mating with winged males. Nevertheless, the paucity of inseminated females in our colony fragments raises the question about the origin of female brood. The possibility of thelytokous parthenogenesis, i.e., the production of female brood from unfertilized eggs, has as yet not been excluded. Morphological queens were absent in the greenhouse and in lab colonies but developed somewhere in the climate chamber after the accidental escape of colony fragments from our nest boxes. This indicates a switch of reproductive strategy from colony growth to dispersal in response to changed environmental conditions, similar to that observed in *Cardiocondyla* (CREMER & HEINZE 2003).

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Zusammenfassung

Die Koloniegründung von *T. vitiensis* MANN, 1921 ist charakterisiert durch das gemeinsame Vorkommen von sich verbreitenden, geflügelten Königinnen und lokalen ungeflügelten Intermorphen, vergleichbar mit anderen Arten der *Technomyrmex albipes* SMITH, 1861 Gruppe. Eine morphometrische Analyse trennte diese beiden Kasten eindeutig auf, wobei die Entwicklungsstufe von Ocelli und Komplexauge deutlich mit der Entwicklung der Ovarien korreliert ist. Intermorphe machen einen beträchtlichen Teil der Kolonie aus. Allerdings war nur ein geringer Teil der Intermorphen unserer Probe befruchtet, obwohl geflügelte Männchen vorhanden waren. Ungeflügelte Männchen, welche in anderen *Technomyrmex*-Arten mit Intermorphen kopulieren, konnten bis jetzt nicht für *T. vitiensis* beschrieben werden. Unsere Daten unterstützen frühere Studien an nah verwandten *Technomyrmex*-Arten und zeigen, dass Reproduktion auf einer opportunistischen Strategie basiert, die es Kolonien ermöglicht, sehr rasch neues Habitat zu besiedeln. Der Ausbruch eines Koloniefragments in die Wand unseres Labors wurde erfolgreich mittels Borsäureködern bekämpft, was darauf hinweist, dass Futter durch Trophallaxis an andere Koloniemitglieder weitergegeben wird.

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