Genetic and social structure of the queen size dimorphic ant *Leptothorax* cf. *Andrei*

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Abstract:

Summary

1. The study of queen polymorphisms can provide insight into the evolution of alternative life histories in ants. In this paper, results of morphological, social and genetic investigations of the newly discovered queen size dimorphism in *Leptothorax* cf. *andrei* are presented.
2. Queens had a bimodal size distribution, and were classified as large (macrogynes) or small (microgynes) queens. Despite their small size, microgynes had a fully developed external flight apparatus and a functional reproductive tract.
3. Queen morphology was not correlated with colony social structure, and the relatedness among nestmate queens was high, indicating secondary polygyny by re-adoption of related queens (daughters) into existing colonies.
4. The distribution of microsatellite alleles indicated that there is a genetic separation between macro- and micro-gynes but the two morphs belong to the same species.
5. The results support the hypothesis that microgyny in *Leptothorax* has not evolved as a specialisation to inter- or intra-specific social parasitism but rather is an adaptation to alternative dispersal behaviour.

KEYWORDS

Body size • dispersal • Leptothorax • life-history evolution • microgynes • queens • reproductive tactic • size polymorphism

Article:

INTRODUCTION

Alternative reproductive tactics

Intraspecific life-history polymorphisms are often coupled with alternative reproductive tactics. Most examples are condition-dependent mating tactics by males: commonly large individuals choose to compete for females and small individuals engage in sneaking tactics (Andersson, 1994; Gross, 1996). In females, variable reproductive morphotypes are less common and may result from mimicry, alternating life-history or dispersal polymorphism (Rüppell & Heinze, 1999). Dispersal polymorphism is relatively common in ant queens because the existing work force of the natal colony makes philopatry rewarding (Nonacs, 1988), and available nesting habitat is often distributed patchily (Bourke & Heinze, 1994). On the other hand, it is always advantageous to parents to have at least some offspring capable of long-range dispersal (

Hamilton Hamilton, W.D. & May R.M. (1977) Dispersal in stable habitats. Nature, 269, 578–581.
) . At least some of these female morphotypes are caused by genetic polymorphism (e.g. Heinze & Buschinger, 1989) and hence are prime examples of variability maintained by frequency-dependent selection or intraspecific adaptation to different habitats.

Reproductive tactics in ant queens

The reproductive tactics of female sexuals (queens) in ants fall into two major categories: independent and dependent colony founding (Hölldobler & Wilson, 1977, 1990; Bourke & Franks, 1995). The ancestral mode of reproduction in ants is probably independent colony founding by a single queen that raises its first worker brood on its own. Dependent colony founding includes social parasitism (usurpation of an unrelated colony by a young queen) and re-adoption of daughter queens into their natal colonies with subsequent colony establishment by budding. The different modes of colony founding are associated with different amounts of body reserves of the foundresses (Keller & Passera, 1989, 1990) and consequently with their body sizes (Stille, 1996). Reduced body sizes of many social parasite queens have been attributed to disguising their identity in order to deceive host workers (Aron *et al*. 1999) but small body size may also relate to the fact that body reserves are not necessary for independent colony founding (Douwes, 1990). Re-adoption into the mother colony allows for a reduction in body size because it circumvents the solitary founding step (Hölldobler & Wilson, 1977; Bourke & Franks, 1995), although size reduction in this context might also play a role in disguising caste fate as a selfish strategy (Bourke & Ratnieks, 1999).

In a few ants, queen size is dimorphic and the smaller queens (microgynes) are almost isometrically reduced copies of the large queens (macrogynes) (Rüppell & Heinze, 1999). In some ants where queens are dimorphic in size, it has been suggested that microgyny might evolve into social parasitism (Bourke & Franks, 1991). Other studies invoked an evolutionary transition to a more pronounced dispersal polymorphism with the loss of wings in one morph (Heinze & Hölldobler, 1993).

The genus *Leptothorax* is rich in social parasites, wing-dimorphic species, and cases of queen size dimorphism (Rüppell & Heinze, 1999). Consequently, case studies in the genus *Leptothorax* are particularly suitable for studying the evolutionary dynamics of queen size dimorphism in ants (Bourke & Franks, 1991). Data are currently available for only two queen size-dimorphic *Leptothorax* species, *L. spinosior* (Hamaguchi & Kinomura, 1996; Hamaguchi *et al*. 1998) and *L. rugatulus* (Rüppell *et al*. 1998). Here, data are presented on the newly discovered queen size dimorphism in *Leptothorax* cf. *andrei* from Mexico, whose origin is presumably phylogenetically independent of those in the other two species. This study considered the morphological differentiation between queen morphs, the consequences of size dimorphism for colony structure, and its prospects for speciation.

MATERIALS AND METHODS

Material

Colonies of *Leptothorax* cf. *andrei* were collected from 19 June to 8 July 1998 at three sites in the Sierra Madre Occidental in western Mexico: 14 colonies 25 km west of San Juanito, Chihuahua province (28°05'N, 107°45'W), 33 colonies 75 km south of Creel, Chihuahua

province (27°15'N, 107°30'W), and 16 colonies 65 km south of Durango, Durango province (23°45'N, 104°30'W). Nests were found in montane forests under stones or in rock crevices. The small size of colonies made their complete collection and immediate census of their queens and workers possible. Colonies were either taken back to the laboratory alive or stored in 96% ethanol for DNA preservation.

Although this yellowish species with 12-segmented antennae was identified as *Leptothorax andrei* (Creighton, 1950), it might be a different, closely related species (A. Francoeur, pers. comm.). Voucher specimens have been sent to P. Ward (University of California, Davies), A. Francoeur (Centre de données sur la biodiversité du Quebec), and S. Cover (Harvard University).

Laboratory methods

Queen head width and thorax width and worker head width were determined to the nearest μm using a micrometer screw table mounted under a Wild^{TM} stereo-microscope (Heerbrugg, Switzerland). After killing the queens by freezing, their reproductive states were assessed by dissection (Buschinger & Alloway, 1978), and their heads and thoraxes were used for DNA extraction (modified from Altschmied *et al*. 1997). Four of 15 microsatellite primer pairs (Table 1) from different *Leptothorax* species (Hamaguchi *et al*. 1993 ; Bourke *et al*. 1997 ; Foitzik *et al*. 1997) were adopted to genotype macro- and micro-gynes (detailed information about the conditions used in the polymerase chain reactions and visualisation are available from the corresponding author on request).

Table 1. Summary statistics of the four microsatellite loci that were used to investigate genetic structure across the five *subpopulations* in three populations of *Leptothorax* cf. *andrei*.

		Number of alleles at loci				
Population	**Sample size**	**L5**	**L18**	**LXGT218**	**LXGT223**	**Average Hexp/Hobs**
Durango (micro)	17	5	13	4	4	0.71/0.75
Durango (macro)	16	4	11	4	5	0.65/0.78
Creel (micro)	22	2	23	9	7	0.75/0.56
San Juanito (micro)	3	2	4	4	5	0.78/0.75
San Juanito (macro)	7	3	8	4	7	0.67/0.64
Overall	65	7	35	13	13	0.85/0.68

The computer program Relatedness 5.0.2 (Goodnight & Queller, 1998) was used to calculate average relatedness of nestmate queens (Queller & Goodnight, 1989) from the genotypic information. Allele frequencies were calculated from individuals using a bias correction for related individuals in the same group as the focal animal. The average relatedness coefficients were obtained by weighting colonies equally.

Genetic differentiation between the morphs was studied by two four-level hierarchical analyses of molecular variance (amova) using the computer program GDA (Lewis & Zaykin, 1999). Two nesting orders were used: individuals in colonies in morphs in populations, and individuals

in colonies in populations in morphs. Both hierarchies were used because, in the absence of *a priori* information on structuring, the correct hierarchy depends on the amount of differentiation at the different levels. The amova analyses were combined with an unweighted pair-group method using arithmetic averages (upgma) cluster analysis using the computer program TFPGA (Miller, 1998). Clustering was based on Reynolds' coancestry coefficient (Reynolds *et al*. 1983) but the tree topology remained robust when Nei's D (Nei, 1972) or Wright's modification of Roger's distance (Wright, 1978), or the computer program GDA was used.

RESULTS

Morphology

The distribution of worker size in *Leptothorax* cf. *andrei* was not significantly different from a normal distribution (Shapiro–Wilk's test: W = 0.96, n = 62, P= 0.06; Fig. 1). In contrast, overall queen size (mean of head width and thorax width) distribution showed a significant deviation from normality (W = 0.91, n = 95, P < 0.001) and conformed better to two overlapping normal distributions (W_{small} = 0.98, n = 68, P= NS and W_{large} = 0.94, n = 27, P= NS). From the observed size distribution of queens (Fig. 1), a head width of 680 µm was selected as the threshold value to classify queens as microgynes (< 680 µm) or macrogynes (≥ 680 µm). Thorax and head width in queens were highly correlated (Spearman's r_s = 0.91, n = 95, P < 0.001) despite a relative increase in thorax width with overall size (thorax:head width ratio correlated positively with the sum of thorax and head width: r_s = 0.84, n = 95, P < 0.001). Worker size did not differ between macrogynous and microgynous colonies (t = 1.31, d.f. = 61, P= NS).

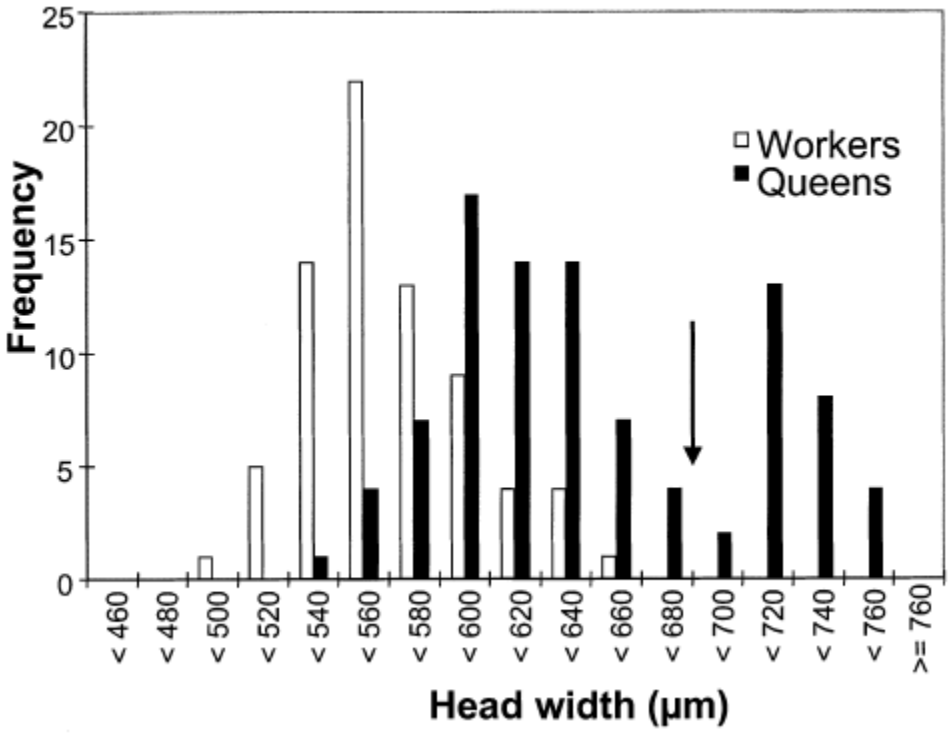

Fig. 1 Frequency distribution of head width in workers and queens of *Leptothorax* cf. *andrei*. The critical value to distinguish macro- from micro-gynes is indicated by an arrow.

Twenty of 21 (95.2%) dissected queens were fertilised and their ovaries were developed to some extent. The number of ovarioles ranged from five to nine, with a median of eight (Fig. 2). Despite the same median for macro- and micro-gynes (eight), macrogynes contained significantly more ovarioles than microgynes (Mann–Whitney $U_{(13,7)} = 28.0$, $p_{(adj)} = 0.02$). Workers had two undeveloped ovarioles and showed no sign of reproduction in colonies with queen presence.

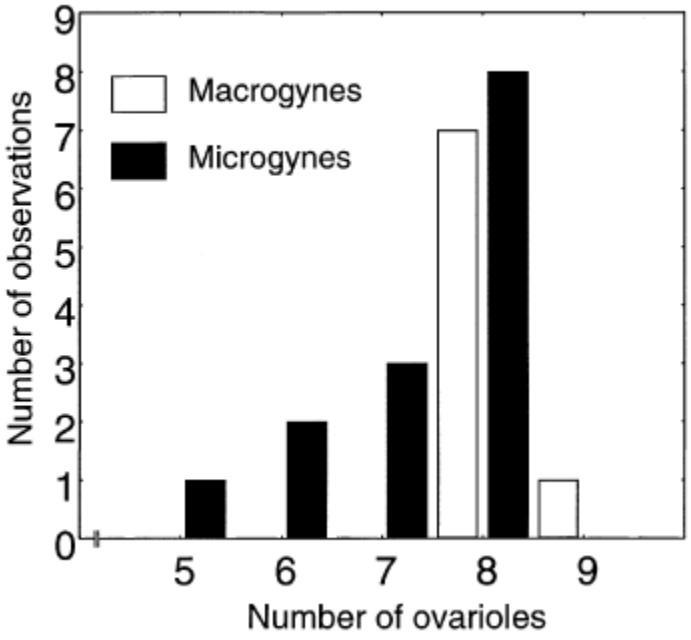

Fig. 2 The number of ovarioles found in dissections of macro- and micro-gynes.

Colony structure

Overall, 69 microgynes occurred in 24 polygynous colonies, and 11 were found in monogynous colonies (mean queen number of all colonies with microgynes = 2.3). Twenty-six macrogynes were found in 10 polygynous colonies and nine in monogynous colonies (mean queen number of all colonies with macrogynes = 1.8). There was no significant relationship between queen morphology and social colony type ($\chi^2 = 2.43$, d.f. = 1, *P*= NS). Six mixed colonies with macro- and micro-gynes were included in this analysis; however from the detailed results in the different populations (Table 2) it is apparent that microgynes occur slightly more often in polygynous colonies than do macrogynes.

Table 2. Colony composition of *Leptothorax* cf. *andrei* from three populations. The numbers of respective colonies and, in brackets, the numbers of queens are given.

Population	Queenless colonies	Colonies with macrogynes		Colonies with microgynes		Colonies with both types of queen (macrogynes/microgynes)	Sample size (macrogynes/microgynes)
		Polygynous	Monogynous	Polygynous	Monogynous		

San Juanito	3	1 (3)	4	1 (2)	3	2 (4/9)	14 (11/14)
Creel	9	0	1	11 (25)	8	3 (4/11)	32 (5/44)
Durango	2	3 (14)	4	6 (21)	0	1 (1/1)	16 (19/22)
Total	14	4 (17)	9	18 (48)	11	6 (9/21)	62 (35/80)

The frequency of microgynes varied among the three populations, from 53.7% at Durango and 56.0% at San Juanito to 89.8% at Creel. Creel also differed in terms of its nest sites (rock crevices vs. nests under loose stones) and its richer vegetation. Further, colony size differed significantly among populations (Kruskal–Wallis test: $H_{(2,61)} = 9.79$, $P < 0.01$), with the smallest size at Creel (average worker number = 25 ± 26 SD), intermediate size at San Juanito (36 ± 19), and largest size at Durango (48 ± 32). The size of a colony was related to the morphology of its queens ($H_{(3,61)} = 10.07$, $P < 0.05$): microgynous (30 ± 27) and queenless (30 ± 23) colonies were smallest, macrogynous colonies were intermediate (36 ± 27), and mixed colonies (with macro- and micro-gynes) were largest (65 ± 21). Colony size was correlated significantly with the number of small queens (multiple regression: $r_{partial} = 0.28$, $t_{(60)} = 2.25$, $P < 0.05$) but not with the number of large queens ($r_{partial} = 0.13$, $t_{(60)} = 1.06$, P= NS).

Genetic data

Descriptive statistics for the four microsatellite loci (Table 1) showed that all loci were polymorphic in each subpopulation. The genetic differentiation was significant at the population level in both hierarchical orders (morphs nested in populations, populations nested in morphs) but between-morph differentiation was only found when morphs were nested within populations (Table 3). Thus, the nesting of morphs in populations is the more appropriate model. Within populations, there was a differentiation of the gene pools between morphs but differentiation at the population level was stronger. The latter result was also obtained by the upgma cluster analysis (Fig. 3). In both populations where macro- and micro-gynes were investigated, the two morph-groups had similar allele distributions and thus clustered together. The clustering distance to queen groupings of the same morph from different populations was consistently larger.

Table 3. Results of the two four-level amovas that were performed to investigate the genetic differentiation between populations and morphs of *L.* cf. *andrei*. Means are given with 95% CI (obtained by bootstrapping over loci). *F*-values are variance components (structuring) at each hierarchical level (F_{IT}: overall inbreeding coefficient, F_{IS}: individual inbreeding within populations), however the significantly negative F_{IS} values are an artefact of the structure of the data set (often only one queen per colony).

Hierarchy	F_{IS}	F_{IT}	F_{Colony}	F_{Morph}	$F_{Population}$
Individuals in colonies in morphs in populations	−0.28 (−0.35 to −0.19)	0.27 (0.05 to 0.45)	0.43 (0.29 to 0.57)	0.21 (0.05 to 0.36)	0.20 (0.02 to 0.39)
Individuals in	−0.28	0.21	0.39	−0.09	0.15

colonies in populations in morphs	(−0.35 to −0.19)	(0.03 to 0.38)	(0.28 to 0.51)	(−0.15 to −0.02)	(0.03 to 0.27)

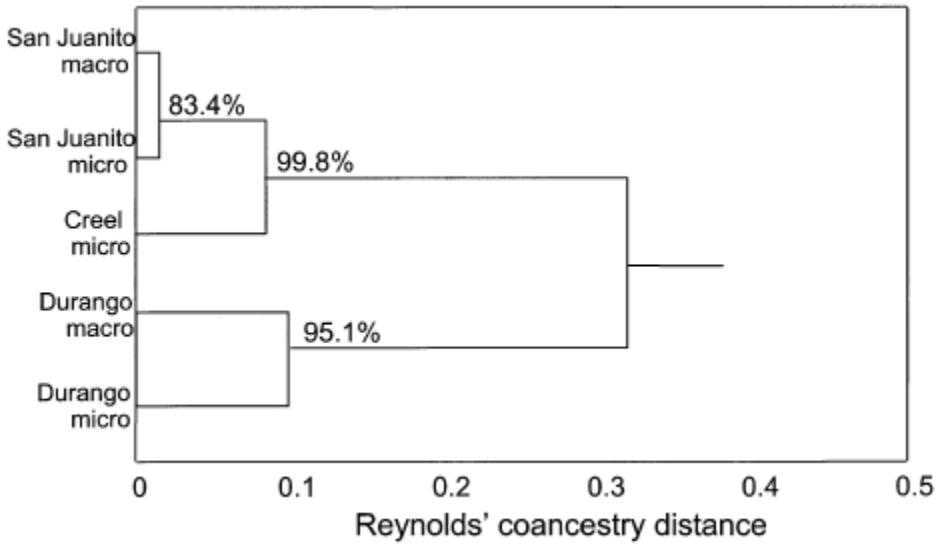

Fig. 3 UPGMA-dendrogram of two macro- and three micro-gyne *subpopulations* on the basis of microsatellite allele frequency distribution. Bootstrap support is given above each node.

Further, the genetic data suggest strong genetic population structuring at the colony level (Table 3). Most queens within colonies were highly related (average intra-colonial relatedness = 0.44) and the values for microgynous and mixed colonies were not significantly different from this overall estimate, whereas macrogynous colonies had an intra-colonial relatedness coefficient higher than the overall value (Table 4).

Table 4. Relatedness coefficients within colonies of *L.* cf. *andrei.* 95% CI and SE were obtained by jackknifing over loci. *n* is the number of colonies.

	All colonies (*n* = 20)	**Macrogynous colonies (*n* = 4)**	**Microgynous colonies (*n* = 13)**	**Mixed colonies (*n* = 4)**
R (± SE)	0.43 ± 0.03	0.64 ± 0.02	0.42 ± 0.05	0.35 ± 0.06
95% CI	0.33–0.53	0.56–0.71	0.28–0.56	0.17–0.54

DISCUSSION

The work reported here describes a new case of queen size polymorphism in ants. Despite some overlap, the bimodal size distribution of queens warrants categorising the queens of *Leptothorax* cf. *andrei* into macro- and micro-gynes. The small thorax width relative to head width in microgynes could be explained in three different ways: (1) The head width might be conserved because of neurological constraints. (2) Microgynes do not need a large pterothorax because of a

smaller wing load (Rüppell *et al*. 1998). (3) Microgynes do not need a large pterothorax because of lower flight activity, which would be evidence for alternative reproductive tactics. Alternative reproductive morphotypes in females are rare (Gross, 1996) but ants are exceptional in this respect (Heinze & Tsuji, 1995; Rüppell & Heinze, 1999). This is further supported by the results on *Leptothorax* cf. *andrei* presented here. A size reduction in this particular case may be possible because selection to maximise individual egg productivity is reduced by the possibility of re-adopting additional queens. Thus, colony productivity, rather than queen productivity, may be optimised and queen number might fine-tune overall egg-laying capacity.

Microgynes are functional queens with slightly less reproductive potential (in terms of number of ovarioles) than macrogynes. The difference in fertility between queen morphs contrasts with findings in other queen dimorphic *Leptothorax* species (Heinze & Buschinger, 1987; Rüppell *et al*. 1998) but has been suggested for the queen size-dimorphic *Ectatomma ruidum* (Schatz *et al*. 1996) and *Polyrhachis* cf. *doddi* (Heinze & Hölldobler, 1993). Overall, female body size is a strong predictor of fertility in insects (Thornhill & Alcock, 1983). The lower ovariole number in microgynes, which presumably reduces their fertility, might explain why microgynous colonies in this study were smaller than macrogynous colonies. It may also explain the relationship between colony size and the number of microgynes: the growth of microgynous colonies might be limited by egg laying capacity, whereas total egg production in macrogynous colonies is not affected significantly by the adoption of additional queens but by the number of workers. An alternative explanation is that (micro-) gyne adoption depends on colony size not vice versa; experimental evidence for both hypotheses is lacking.

Microgyny is generally viewed as an adaptation to dependent colony founding in ants (Bourke & Franks, 1995; Rüppell & Heinze, 1999), and good evidence for this exists in some species (McInnes & Tschinkel, 1995; O. Rüppell *et al*. unpublished). For *Leptothorax* cf. *andrei*, however, a close link between queen body size and colony structure could not be demonstrated, which would have provided strong support for this hypothesis.

Approximately 14% of the microgynes were found in monogynous colonies, which may be explained either by some independent colony founding by microgynes or by frequent colony budding. The latter hypothesis seems more plausible because no newly initiated colonies could be found, and with regard to nesting substrate, colonies were patchily distributed in high quality locations surrounded by unsuitable habitat. Furthermore, budding is a common reproductive tactic in *Leptothorax* ants (Bourke & Franks, 1995).

The high relatedness coefficients indicate clearly that most polygynous colonies arose by re-adoption of related queens, irrespective of morphology. Colonies comprise a complex mixture of queen–queen relatedness values. The significantly higher relatedness coefficient in macrogynous colonies indicates that most relationships in these colonies are between mothers and daughters and between full sisters, however it has to be acknowledged that microgynes and even macro- and micro-gynes in mixed colonies are also related to some degree.

Overall, the general conclusion of secondary polygyny by re-adoption in *Leptothorax* cf. *andrei* is well supported. As in other studies of queen size-dimorphic *Leptothorax* species (Hamaguchi *et al*. 1998 ; O. Rüppell *et al*. unpublished), it cannot be excluded that occasional adoption of

unrelated queens occurs but social parasitism seems not to be the prevalent reproductive tactic in microgynes and thus not a factor responsible for their evolution.

These results parallel findings in *Leptothorax rugatulus* (O. Rüppell *et al*. unpublished) that macro- and micro-gynes should be considered the same species, in spite of a genetic separation of their gene pools. The genetic separation, also manifested in the lower relatedness within mixed colonies, indicates different mating tactics by macro- and micro-gynes and the potential for sympatric speciation (Buschinger, 1990; Bourke & Franks, 1991). Microgynes might mate locally, while macrogynes join mating swarms (leks), an observation already made in *Myrmica* (Elmes, 1991). Nevertheless, gene flow via males constitutes a potent mechanism in ants with philopatric females to prevent inbreeding and consequently speciation (Bourke & Franks, 1995).

Like macrogynes of *L. rugatulus* (O. Rüppell *et al*. unpublished) or queens of conventional facultatively polygynous ant species, *Leptothorax* cf. *andrei* macrogynes probably switch between independent colony initiation and returning to their natal colonies, depending on environmental conditions. Thus, two different reproductive tactics, independent colony founding and re-adoption, co-exist, and some adaptive size modifications have evolved, however behavioural responses to environmental cues are more flexible and are consequently only correlated loosely with morphotype.

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