

Morphological variability of intercastes in the ant *Temnothorax nylanderi*: pattern of trait expression and modularity

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Received: 2 November 2012 / Revised: 6 March 2013 / Accepted: 6 March 2013 / Published online: 22 March 2013
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Abstract Ants have distinct morphological castes (queens and workers), but aberrant queen-worker “intercastes” occasionally occur, both in wild and laboratory conditions. Intercastes are rare, however, such novel phenotypes may have evolutionary significance. Their morphology is highly variable in any given species, providing valuable information about the integration of queen traits (e.g. ocelli, wings, complex segmentation of thorax, large gaster and ovaries, spermatheca). Generally, these traits are all diminished or absent in workers. We used multivariate morphometry to analyze an exceptionally large sample of 101 intercastes of *Temnothorax nylanderi*. We determined distributions and correlations of traits, and confirmed the mosaic nature of intercastes. Queen-specific traits are not expressed coherently in intercastes, but the possible patterns of trait combination are limited. A large number of small-sized intercastes had disproportionately larger head, ocelli and gaster but smaller thorax. In contrast, queen-like growth of thorax and rudimentary wings only occurred in large-sized intercastes. This is the most comprehensive analysis of

intercaste variability, and suggests the existence of constraints on recombination of caste-specific modular traits.

Keywords Intercaste · Modularity · Morphological integration · Polyphenism

Introduction

In a proportion of eusocial insects, reproductive division of labor is based on morphological specialization among colony members. This is most pronounced in ants because workers always lack wings and often show greatly reduced organs for vision and reproduction. Queens generally have well-developed organs for flight and reproduction, reflecting adaptations for dispersal, colony founding and prolific egg-laying (Hölldobler and Wilson, 1990; Wheeler, 1986). Molet et al. (2012) argued that modularity is a key feature of caste dimorphism in social Hymenoptera. Modules are sets of traits that are internally integrated but relatively independent from other modules (Klingenberg, 2008). Queens and workers express differently some modular traits such as flight thorax, ovaries, spermatheca, and thus caste-specific traits can evolve independently. During normal ontogeny, female larvae develop into either of two discrete castes due to different levels of signals such as JH and insulin-like growth factor (Nijhout and Wheeler, 1982; Nijhout, 1994; Hartfelder and Emlen, 2005).

The existence of morphological anomalies that are intermediate between the queen and worker phenotypes has long been known (e.g. Wheeler, 1937). “Intercastes” are rare individuals that have accidentally deviated from normal developmental pathways (Peeters, 1991; Heinze, 1998; Molet et al., 2012). Intercastes are particularly conspicuous in ants due to the winged/wingless polyphenism. They occur

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naturally in the wild (Düssmann et al., 1996; Kikuchi et al., 1999; Molet et al., 2009; Plateaux, 1970). In addition, they can be induced by artificial perturbations of development (Plateaux, 1970; Murakami et al., 2002) or by parasite infections (Csösz and Majoros, 2009). Although intercastes survive as adults, their function and behaviour are usually unstudied. Most reports of intercastes are anecdotal but in *Temnothorax nylanderi* (subfamily Myrmicinae), Plateaux (1970) assembled a large sample of >100 intercastes and showed their morphology to be highly variable, with apparently uncoordinated expression of visual, flight and reproductive organs. Some body parts of intercastes resembled those of winged queens, whereas other body parts resembled those of workers. In other words, the normal combinations of queen- and worker-specific modules were broken. Molet et al. (2012) postulated that novel combinations of modules in intercastes can be selected and evolve into regularly produced castes. However, how caste-specific modules are shuffled to give a queen-worker mosaic is largely unknown.

Using multivariate morphometry, we examined a large sample of *T. nylanderi* intercastes distinct from those studied by Plateaux (1970). We analyzed the pattern of trait expression and the levels of correlation with principal component analysis (PCA). The intercastes of *T. nylanderi* apparently have no function in their colonies, but we discuss the potential evolutionary significance of novel intercaste phenotypes.

Materials and methods

Biological material

Temnothorax nylanderi is monogynous with a strong queen–worker dimorphism. Workers are monomorphic, and they have ovaries but lack a spermatheca (Plateaux, 1970). Individuals with vestigial ocelli, unusual shape of thorax, aberrant thoracic segmentation, or vestigial wings were categorized as intercastes. A sample of 101 intercastes was obtained from wild and laboratory-reared colonies of *T. nylanderi* collected in Fontainebleau, Bruyères-le-Chatel and Forêt de Bercé, France, from 1958 to 1987. All intercastes studied here were different from those in Plateaux (1970). Hundreds of colonies were reared in the laboratory for 10 or more years, with warm conditions (24 °C, sometimes 20–26 °C) alternating with overwintering (about 5 °C). Several large colonies produced up to 10–20 intercastes at the same time as winged queens. This co-occurrence of queen and intercaste was apparently due to decreased growth just before metamorphosis of queen-destined larvae (Plateaux, 1970). Note that the large number of intercastes available for study does not mean they are common in

T. nylanderi. Hundreds of colonies collected by L. Plateaux did not have any intercastes. Intercastes were also rare in field colonies of *T. affinis*, *T. albipennis*, *T. parvulus* and *T. unifasciatus*. Since the number of intercastes available from any one colony was limited, individuals from 29 colonies were lumped together for morphological analysis [10 intercastes from 4 field colonies, 85 intercastes from 23 laboratory-reared colonies; unknown origins for 6 remaining intercastes ($n = 2$ colonies)]. We used 48 queens and 35 workers for comparison.

Measurements and categorization of traits

All intercastes were photographed. Eight body traits (head width at eye level, eye length, thorax length, thorax height, prothorax width, mesothorax width, length of first gaster segment, gaster width, Fig. 1) were measured using image analysis software Image J (NIH). Another three external traits (ocelli, thoracic segmentation, wings) were categorized arbitrarily. We used four categories of ocelli development: (1) no ocelli; (2) trace of ocelli as cuticular pigmentation; (3) non-protruding ocelli; (4) ocelli protruding above cuticle. Thoracic segmentation was assessed by comparisons with workers and queens: (1) sclerites are completely fused; (2) sclerites are distinct although segmentation is partial and/or unclear; (3) all sclerites are distinct. Categories of wing development were: (1) no wings; (2) wing bud without membranous structure; (3) short wing with membranous structure; (4) fully developed wing.

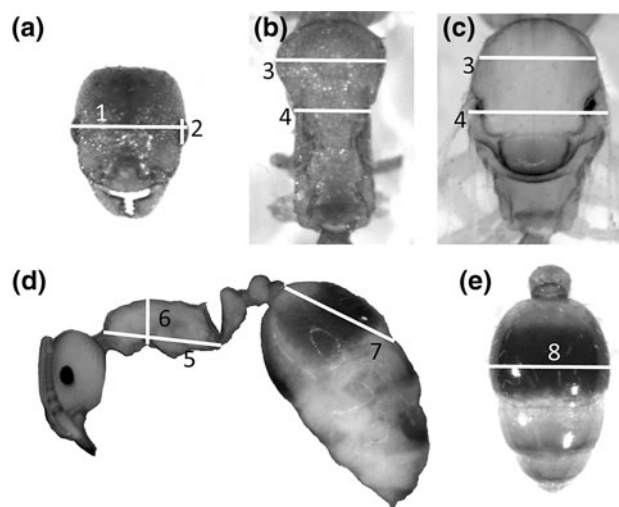


Fig. 1 Body parts measured in *Temnothorax nylanderi*. Worker (a, b, d, e), Queen (c). 1 head width, 2 eye length, 3 prothorax width (b worker, c queen), 4 mesothorax width (b worker, c queen), 5 thorax length, 6 thorax height, 7 gaster length, 8 gaster width

Statistical analyses

The normal distribution of traits was evaluated with Shapiro–Wilk test. If normality was rejected, we fitted a bimodal model (combination of two normal distributions) and calculated an AIC to see which model (monomodal or bimodal) fits better the data (see Akaike, 1974; Miyazaki et al., 2010). Then, we analyzed correlations among the traits. Pearson product moment correlation coefficients (r) were calculated for the eight continuous variables (head width, eye length, thorax length, thorax height, prothorax width, mesothorax width, gaster length, gaster width). Spearman rank correlation coefficients were calculated for the three categorical variables (ocelli development, thoracic segmentation, wings).

Correlation coefficients represent how one trait is dependent on another. However, when there are outliers or a bimodal distribution, simple comparison of coefficients is not a good assessment of phenotypic variability. Thus, to evaluate the relationship of two body parts in another way, the dispersion of points around the regression line was quantified (Eberhard et al., 1998). As an indicator of the accuracy with which fitted regression function predicts the dependence of Y on X , the square root of the mean square of residual from the regression line was calculated for all trait combinations (“standard error of estimate”, Zar, 1984 p 351; Eberhard et al., 1998). Since this indicator is affected by trait size, it was divided by the means of focal trait Y . This normalization allows us to compare magnitudes of dispersion independent of trait values. Result scores were shown as 100-fold values to increase readability (Table 4).

Third, we evaluated the patterns of trait combination and overall morphological variation. Principal component analysis (PCA) was performed using 11 parameters (8 quantitative traits, 3 categorical traits) from queens, workers and intercastes. For PCA, a correlation matrix was used to normalize all parameters with an average of 0 and a variance of 1 (Jolliffe, 2002). JMP 5.1 (SAS) was used for statistical analysis.

Results

Trait distributions

To visualize morphological characteristics of queens, workers and intercastes, representative traits were plotted against head size (Fig. 2). To help comprehend their variability, intercastes were divided into three post hoc categories (IC1, IC2 and IC3) on the basis of thoracic segmentation. This trait alone was a good representation of the overall variations in PC1–PC2 dimensions (see Fig. 3b; “Thoracic segmentation and subgrouping”). Among queens

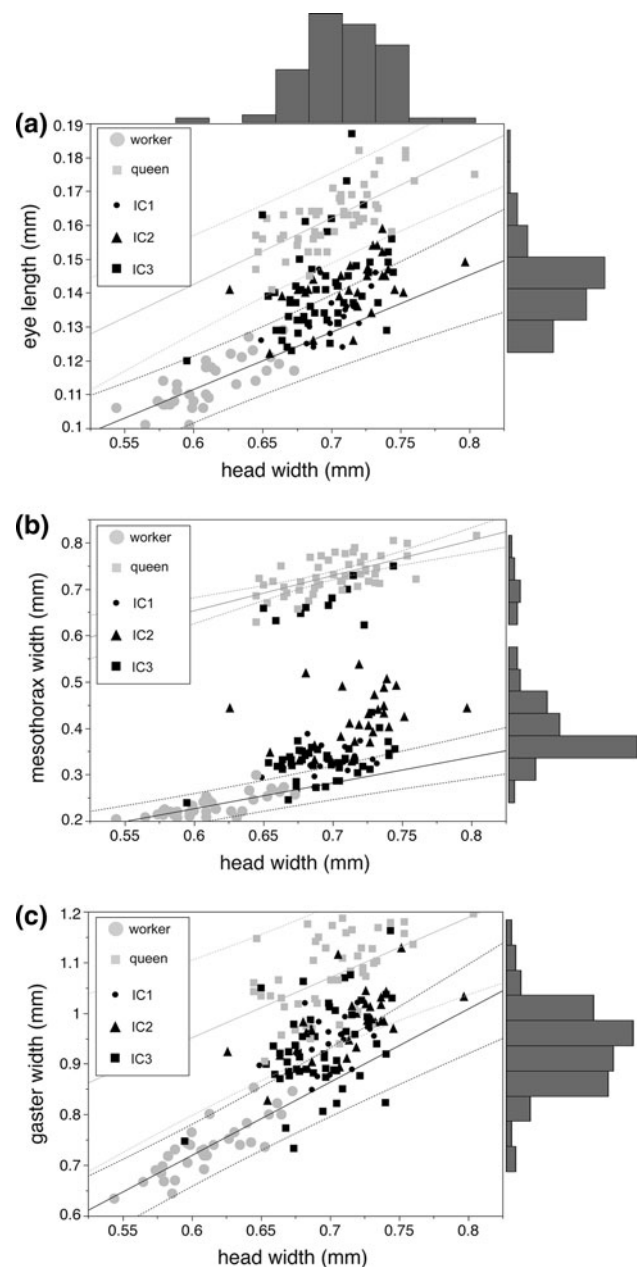
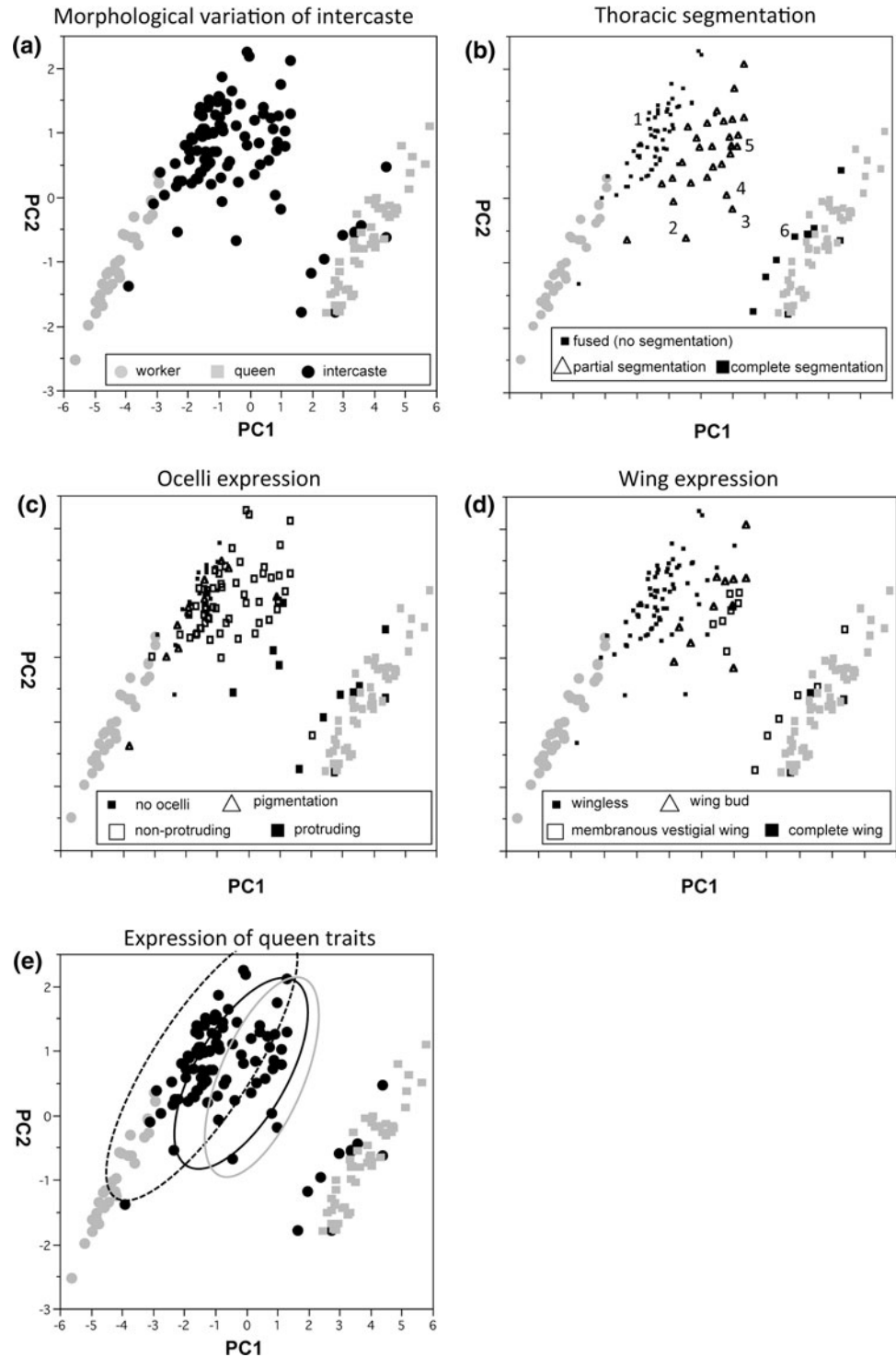


Fig. 2 Allometric relationship of body parts in queens, workers and intercastes of *Temnothorax nylanderi*. Gray square queen ($n = 48$); gray circle worker ($n = 35$). Intercastes were divided into three subcategories based on thoracic segmentation (see “Results” for details). Small black circle IC1 (intercaste with unsegmented thorax, $n = 62$); black triangle IC2 (intercaste with partially segmented thorax, $n = 29$); black square IC3 (intercaste with fully segmented thorax, $n = 10$). Regression lines and 95 % predicted intervals for queen and worker are shown in gray and black, respectively. Histograms indicate size-frequency distribution of intercastes

and workers, all trait sizes exhibited different distributions. Except for head width, ranges of traits did not overlap across castes. All trait distributions can be regarded as normal except mesothorax width of worker, for which Shapiro–Wilk test rejected normality [Table 1; neither monomodal nor

Fig. 3 Morphological variability among intercastes analyzed by principal component analysis (PCA). **a** All-trait morphological variation; **b** variability of thoracic segmentation; **c** variability of ocelli development; **d** variability of wing development. Numbers in **b** (1–6) correspond to SEM photographs in Fig. 4; **e** variability of expression of queen-specific characters, 95 % probability ellipses were depicted for ocelli pigmentation (*dotted ellipse*), thoracic segmentation (*solid ellipse*), wing bud (*gray ellipse*)



bimodal models are better (AICs = -158.8 and -159.9 , respectively)]. Among intercastes, distributions of three thoracic traits (thorax height, prothorax width, mesothorax width) exhibited bimodal patterns (mesothorax width in Fig. 2b, data not shown for thorax height and prothorax width). Shapiro–Wilk test revealed that distribution of these traits differed significantly from normal, and AICs indicated

bimodality (Table 1). In contrast, five traits (thorax length, head width, eye length, gaster width and gaster length) appeared to have unimodal distributions (head width and gaster width in Fig. 2a and c, other data not shown). Among these, Shapiro–Wilk test revealed that head width and gaster width/length did not differ from normal distribution, but eye length and thorax length differed significantly. AICs

Table 1 Shapiro–Wilk tests for normal distribution

	Intercaste				Queen		Worker	
	W	P	AIC (monomodal)	AIC (bimodal)	W	P	W	P
Head width	0.981	0.145	−418.0	−415.2	0.974	0.339	0.97	0.478
Eye length	0.946	0.0004	−613.7	−617.2	0.984	0.719	0.958	0.234
Prothorax width	0.83	<0.0001	−317.1	−377.6	0.971	0.25	0.972	0.535
Mesothorax width	0.791	<0.0001	−151.8	−221.7	0.987	0.864	0.891	0.003
Thorax height	0.826	<0.0001	−284.0	−338.2	0.98	0.536	0.962	0.285
Thorax length	0.943	0.0003	−282.0	−291.1	0.979	0.507	0.958	0.221
Gaster width	0.988	0.53	−230.7	−221.9	0.957	0.068	0.975	0.625
Gaiter length	0.988	0.486	−243.4	−241.1	0.972	0.268	0.974	0.585

Bold letters indicate better fit of the model (lower AIC)

indicated that distributions of eye length and thorax length were slightly closer to bimodal, but the two models did not differ much (Table 1). Plotting eye vs. head (Fig. 2a), about half of intercastes plotted inside the worker 95 % prediction intervals and thus had a worker-like phenotype ($n = 54$), while 11 intercastes were queen-like (i.e. inside the queen 95 % prediction intervals). The remaining individuals ($n = 36$) were intermediate. Plotting mesothorax vs. head (Fig. 2b), most intercastes were intermediate ($n = 73$, 73 %), and the rest were worker-like ($n = 19$, 19 %) or queen-like ($n = 9$, 9 %). Although a majority of intercastes fell in the middle, most of them were clearly closer to the worker phenotype, resulting in bimodality (Fig. 2b). This pattern also resulted from plotting prothorax width and thorax height vs. head width (data not shown). When plotting gaster width vs. head, queen and worker 95 % prediction intervals were very close and sometimes overlapped slightly (Fig. 2c). Most intercastes resembled queens ($n = 57$), and others resembled workers ($n = 33$); the remaining individuals were ambiguous. In summary, different traits varied in their distribution patterns across intercastes.

Trait correlation and dispersion around regression

All correlation coefficients among traits were positive, and they were statistically significant in almost all parameter combinations in intercastes, as well as in queens and workers (Table 2). Therefore, most trait values are positively associated with each other in intercastes, queens and workers.

Another indicator of variability, dispersion around the regression line (i.e. standard error of estimate) is shown in Table 4. Out of 56 trait combinations, intercastes had greater values than queens in 48 combinations, and greater than workers in 53 combinations, consistent with the great variability of intercaste phenotypes. Among intercaste traits, prothorax width, mesothorax width and thorax height exhibited great dispersion around the regression (scores exceeded 10 in 11 combinations), and mesothorax had the

greatest variability. This means that thorax size varies unpredictably in intercastes, hence thorax is less likely to be predicted from other body traits.

Principal component analysis (PCA)

Combination dynamics of multiple traits were analyzed by PCA. First principal component (PC1) explained 82.1 % of total variation, and it was positively correlated with all traits (Table 3), suggesting that PC1 indicates overall body size. In addition, head width had a smaller factor loading (0.216) on PC1 compared to other traits (0.291–0.322). This means that trait growth is not uniform with PC1 increase, and that head width is less correlated to the overall body size than other parameters. In summary, PC1 represents size and shape difference because individuals with large PC1 have large overall size with relatively large thorax and abdomen. PC2 explained 10.1 % of total variation, and it was positively loaded with head width, eye size, gaster width and gaster length, and negatively loaded with several thoracic traits (prothorax width, mesothorax width, thorax height, thoracic segmentation, wing development, Table 3). Thus, trait combinations in PC2 indicate shape variation because large PC2 corresponds to large head, eye and gaster, with relatively small thorax, reduced thoracic segmentation and wing structure.

Individual PC2 scores were plotted on PC1 scores to show individual phenotypes in morphospace (Fig. 3a). Queens and workers had different PC1 scores, reflecting the large overall size of queens, with relatively large thorax and ocelli compared to workers (Fig. 2b, c). Distribution of intercaste plots in PC1–PC2 morphospace indicated that intercaste phenotypes roughly fall into two groups: small (large PC2) and large (small PC2) (Fig. 3a). Under the postulate that intercastes are intermediate in morphology between queens and workers, they would have intermediate PC1 and PC2 scores, and would plot midway between queens and workers. However, most of the small-sized

Table 2 Correlation coefficients between traits (* $p < 0.05$)

Intercaste	HW	EL	PW	MW	TH	TL	GW	GL	OD	TS	WD
Head width (HW)		0.38*	0.30*	0.21	0.19	0.50*	0.51*	0.55*	0.02	0.20*	0.27*
Eye length (EL)	0.38*		0.70*	0.71*	0.74*	0.67*	0.52*	0.52*	0.49*	0.52*	0.60*
Prothorax width (PW)	0.30*	0.70*		0.93*	0.89*	0.88*	0.58*	0.48*	0.38*	0.64*	0.66*
Mesothorax width (MW)	0.21	0.71*	0.93*		0.92*	0.88*	0.53*	0.46*	0.55*	0.75*	0.71*
Thorax height (TH)	0.19	0.74*	0.89*	0.92*		0.83*	0.59*	0.46*	0.46*	0.67*	0.61*
Thorax length (TL)	0.50*	0.67*	0.88*	0.88*	0.83*		0.68*	0.62*	0.37*	0.59*	0.66*
Gaster width (GW)	0.51*	0.52*	0.58*	0.53*	0.59*	0.68*		0.72*	0.28*	0.48*	0.47*
Gaster length (GL)	0.55*	0.52*	0.48*	0.46*	0.46*	0.62*	0.72*		0.24*	0.42*	0.38*
Ocelli development (OD)	0.02	0.49*	0.38*	0.55*	0.46*	0.37*	0.28*	0.24*		0.60*	0.56*
Thoracic segmentation (TS)	0.20*	0.52*	0.64*	0.75*	0.67*	0.59*	0.48*	0.42*	0.60*		0.77*
Wing development (WD)	0.27*	0.60*	0.66*	0.71*	0.61*	0.66*	0.47*	0.38*	0.56*	0.77*	
Queen	HW	EL	PW	MW	TH	TL	GW	GL			
Head width (HW)		0.71*	0.45*	0.64*	0.41*	0.67*	0.50*	0.70*			
Eye length (EL)	0.71*		0.22	0.34*	0.21	0.45*	0.23	0.47*			
Prothorax width (PW)	0.45*	0.22		0.69*	0.50*	0.46*	0.55*	0.49*			
Mesothorax width (MW)	0.64*	0.34*	0.69*		0.61*	0.73*	0.80*	0.70*			
Thorax height (TH)	0.41*	0.21	0.50*	0.61*		0.51*	0.63*	0.49*			
Thorax length (TL)	0.67*	0.45*	0.46*	0.73*	0.51*		0.71*	0.74*			
Gaster width (GW)	0.50*	0.23	0.55*	0.80*	0.63*	0.71*		0.71*			
Gaster length (GL)	0.70*	0.47*	0.49*	0.70*	0.49*	0.74*	0.71*				
Worker	HW	EL	PW	MW	TH	TL	GW	GL			
Head width (HW)		0.75*	0.88*	0.75*	0.61*	0.84*	0.85*	0.82*			
Eye length (EL)	0.75*		0.80*	0.54*	0.36*	0.55*	0.57*	0.56*			
Prothorax width (PW)	0.88*	0.80*		0.81*	0.62*	0.81*	0.76*	0.65*			
Mesothorax width (MW)	0.75*	0.54*	0.81*		0.63*	0.77*	0.75*	0.69*			
Thorax height (TH)	0.61*	0.36*	0.62*	0.63*		0.58*	0.68*	0.47*			
Thorax length (TL)	0.84*	0.55*	0.81*	0.77*	0.58*		0.87*	0.77*			
Gaster width (GW)	0.85*	0.57*	0.76*	0.75*	0.68*	0.87*		0.85*			
Gaster length (GL)	0.82*	0.56*	0.65*	0.69*	0.47*	0.77*	0.85*				

intercastes (large PC2) plotted above both queens and workers. This means that a large number of small-sized intercastes had disproportionately larger head and gaster with smaller thorax, which is not expected from a queen-worker intermediate.

Thoracic segmentation and subgrouping of intercastes

Different degrees of thoracic fusion clearly subdivided small-sized intercastes into two groups in the PC1–PC2 morphospace (Fig. 3b). Thus, intercastes were classified

Table 3 Factor loadings of principal component analysis (PCA)

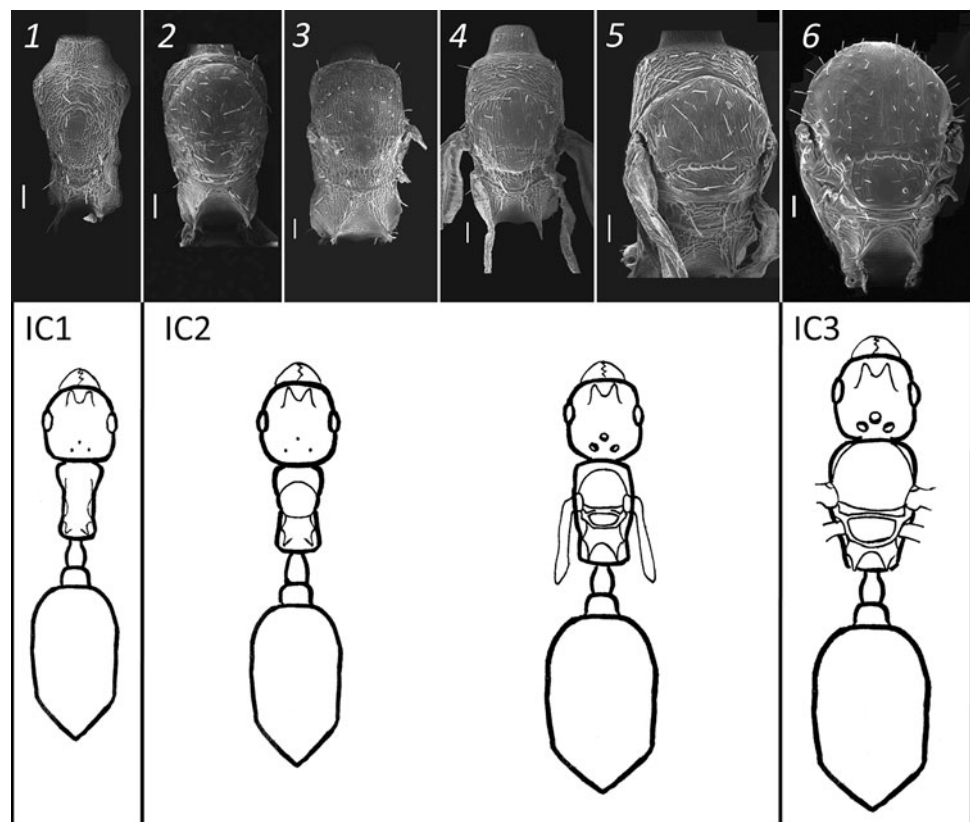
	PC1 (82.1 %)	PC2 (10.1 %)
Head width (HW)	0.216	0.678
Eye length (EL)	0.312	0.112
Prothorax width (PW)	0.322	-0.118
Mesothorax width (MW)	0.322	-0.212
Thorax length (TL)	0.325	0.055
Thorax height (TH)	0.318	-0.169
Gaster length (GL)	0.295	0.343
Gaster width (GW)	0.300	0.282
Ocelli development (OD)	0.291	-0.064
Thoracic segmentation (TS)	0.302	-0.332
Wing structure (WS)	0.298	-0.356

into three post hoc categories: unsegmented thorax (IC1, small squares in Fig. 3b, $n = 62$), partially segmented thorax (IC2, triangles in Fig. 3b, $n = 29$) and fully segmented thorax (IC3, large squares in Fig. 3b, $n = 10$). Representatives of these three intercaste categories are detailed in Fig. 4, with corresponding individual plots in Fig. 3b (#1–6). The thoracic tergites of IC1 (Fig. 4, #1) were fused and indistinguishable from those of workers. In contrast, IC2 had distinct promesonotal groove, mesonotum and/or scutellum (Fig. 4, #2–5). The metanotum–propodium groove

was also often visible in IC2. Interestingly, thoracic morphology and segmentation pattern of IC2 were highly variable, including queen-like phenotype (Fig. 4, #2), worker-like phenotype (Fig. 4, #3) and intermediates (Fig. 4, #4, 5). IC3 (Fig. 4, #6) had a big mesonotum projecting over the pronotum, while scutellum and metanotum–propodium groove were distinct as in queens.

IC1 (intercaste with unsegmented thorax) had larger PC1 and PC2 than workers, indicating larger overall size and larger head and gaster. Indeed, the sizes of head, eye and gaster in IC1 frequently reached size ranges of queens (Fig. 2a, c). In addition, IC1 frequently had distinct ocelli (Fig. 3c and see below), and this may also account for their increased PC2. IC2 (intercaste with partially segmented thorax) had larger PC1 (i.e. overall size) than IC1, indicating that IC2 were bigger than IC1. The majority of IC2 had PC2 scores similar to IC1, but some had lower scores (e.g. #2, 3, 4 in Fig. 3b; Fig. 4). Low PC2 can be interpreted to be large thorax or small eye and/or gaster. In scatter plots of raw data, IC2 is characterized by large thorax rather than small eye and gaster (Fig. 2a–c). Thus, lowered PC2 in IC2 is interpreted as increased growth of the thorax. Plots of IC3 (intercaste with fully segmented thorax) almost overlapped with those of queens, PC1 was larger than in IC2, and PC2 was smaller. This indicates that IC3 is bigger in overall size, with a larger relative thorax size.

Fig. 4 SEM photographs and schematic illustrations of *Temnothorax nylanderi* intercastes numbers 1–6 correspond to the numbers in Fig. 3b. 1 intercaste with unsegmented thorax (IC1); 2–5 intercaste with partially segmented thorax (IC2); 6 intercaste with fully segmented thorax (IC3). Bars indicate 100 μ m



Ocelli expression

Winged queens have three ocelli involved in stabilization reflexes during flight, and each consist of photoreceptors together with a large lens protruding above the cuticle. Ocelli were frequently expressed in IC1, despite a lack of both wings and flight thorax. Most IC1 had pigmented spots (=traces of photoreceptors) or a distinct but non-protruding lens (Fig. 3c). In IC2, most individuals had non-protruding ocelli. In all IC3 except one, ocelli were protruding structures as in queens (Fig. 3c).

Wing expression

Wings never developed in IC1 (Fig. 3d). In contrast, most IC2 individuals had wing buds and some had membranous vestigial wings. All IC3 had membranous wings (Fig. 3d).

Onset of queen-trait expression

To visualize the variation of queen-trait expression, 95 % confidence ellipses of three traits (ocelli pigmentation, thoracic segmentation, wing bud) were depicted in PC1–PC2 morphospace (Fig. 3e). Ocelli pigmentation ellipse was close to workers (Fig. 3e, dotted ellipse). In contrast, thoracic segmentation and wing bud ellipses were closer to queens (Fig. 3e, solid and gray ellipses).

Discussion

Morphological variability of intercastes

Our data confirm the considerable morphological heterogeneity documented by Plateaux (1970) using a distinct large sample of *T. nylanderi* intercastes. The bimodal distributions of thoracic size and PC1 (overall size and shape) in intercastes indicated that most of them are either worker-like or queen-like (Figs. 2b, 3a). This polarity suggests that the two original developmental pathways (i.e. queen and worker) are fairly robust, and large deviations from these alternative pathways are rare. Based on PC1 (overall size and shape), PC2 (shape) and thoracic segmentation, intercastes can be classified into three subgroups: unsegmented thorax (IC1), partially segmented thorax (IC2), and fully segmented thorax (IC3). Sizes of head, eye and gaster of IC1 and IC2 frequently approximated that of queen (Fig. 2a, c), suggesting that growth of head, eye and gaster can be maximal despite incomplete development of thorax. In contrast, the size of thoracic traits (prothorax width, mesothorax width, thorax height) of IC1 and IC2 seldom reached the size ranges of queen (Fig. 2b). Proportion of thorax size to head size in IC1 was similar to workers', while

proportions in some IC2 and all IC3 were much closer to queens', suggesting that a queen-like thorax develops only in IC2 and IC3 (Fig. 2b). In our sample, about half of intercastes were IC1 and were close to workers in trait scatter plots (Fig. 2a–c) and PC1–PC2 morphospace (Fig. 3b). Since IC1 had worker-like size and shape and most had ocelli, IC1 can be characterized as “large workers with ocelli” (Fig. 3c, #1 in Fig. 4). In contrast, plots of IC3 almost overlapped those of queens, implying that these individuals failed to develop fully as queens (Fig. 3a, #6 in Fig. 4), probably due to nutritional shortage or perturbation during larval development. IC2 had a variety of transitional phenotypes between workers and queens, thus they represent mosaics of these two castes. Especially, wing and thorax phenotypes are quite variable in IC2, and it was almost impossible to predict these from individual size (PC1) or shape (PC2) (Fig. 3d, #2–5 in Fig. 4).

Coherence of queen-specific characters

Plateaux (1970) argued that several queen-specific traits are expressed without coordination in intercastes. Our morphometric analyses did not support this idea entirely, because strong correlation coefficients existed between several traits, both quantitative and qualitative (Table 2). We suggest that development of queen characters is generally coordinated with each other. However, these correlations are not surprising because our sample of intercastes contained both worker-like and queen-like intercastes; our very ability to recognize these is due to the coordinated expression of queen characters.

In order to detect phenotypic variation, dispersion from the regression line was analyzed (Table 4). Dispersion of thoracic sizes (mesothorax width and thorax height) was conspicuous in intercastes (Table 4), suggesting that thorax growth is more independent of other traits. Such great variability of thorax is compatible with Plateaux (1970)'s idea of independence among different traits in intercastes. Some IC2 exhibited disproportionately large thorax and this may contribute to thorax variability in intercastes. In addition, there was a distinct order of queen trait expression (ocelli, thoracic segmentation and wing bud) in PC1–PC2 morphospace (Fig. 3e); ocelli formation is close to worker plots, wings developed close to queen plots, and thorax segmentation is in between. This means that the propensity for expressing queen traits varies among traits, and the possible combinations of queen-specific traits are limited. To summarize, different traits behave as independent modules, but they are expressed in a hierarchical manner, and the combinations of modules are constrained. Plateaux (1970) also compared number of ommatidia (i.e. visual acuity) across castes; intercastes had intermediate numbers but this was not correlated with body size.

Table 4 Standard errors of estimate. Large scores indicate great dispersion around the regression line

Intercaste response variable	predictor variable							
	HW	EL	PW	MW	TH	TL	GW	GL
Head width (HW)		4	4.1	4.2	4.2	3.7	3.7	3.6
Eye length (EL)	7.6		5.8	5.7	5.5	6	6.9	7
Prothorax width (PW)	10.3	7.7		3.9	4.7	5.1	8.8	9.5
Mesothorax width (MW)	28.5	20.3	10.4		11	13.5	24.6	25.8
Thorax height (TH)	19.5	13.3	8.7	7.5		11	15.9	17.6
Thorax length (TL)	5.8	4.9	3.2	3.1	3.7		4.9	5.3
Gaster width (GW)	6.9	6.9	6.5	6.8	6.5	5.9		5.6
Gaster length (GL)	6.5	6.7	6.9	7	7	6.2	5.4	

Queen response variable	predictor variable							
	HW	EL	PW	MW	TH	TL	GW	GL
Head width (HW)		3.4	4.3	3.7	4.4	3.6	4.2	3.5
Eye length (EL)	4.1		5.6	5.4	5.6	5.2	5.6	5.1
Prothorax width (PW)	4.8	5.3		3.9	4.7	4.8	4.5	4.7
Mesothorax width (MW)	4.2	5.2	3.9		4.3	3.7	3.3	3.9
Thorax height (TH)	6.2	6.7	6	5.5		5.9	5.4	6
Thorax length (TL)	3.9	4.6	4.6	3.5	4.4		3.6	3.5
Gaster width (GW)	6.5	7.3	6.3	4.5	5.8	5.3		5.2
Gaster length (GL)	4.6	5.7	5.6	4.6	5.7	4.4	4.5	

Worker response variable	predictor variable							
	HW	EL	PW	MW	TH	TL	GW	GL
Head width (HW)		3.5	2.5	3.5	4.2	2.9	2.8	3.1
Eye length (EL)	4.2		3.9	5.4	6	5.4	5.3	5.3
Prothorax width (PW)	3	3.8		3.7	5	3.8	4.2	4.8
Mesothorax width (MW)	6.8	8.7	6		8	6.6	6.9	7.5
Thorax height (TH)	7.1	8.3	7	6.9		7.3	6.5	7.9
Thorax length (TL)	2.5	3.9	2.7	2.9	3.8		2.3	2.9
Gaster width (GW)	4	6.2	4.9	5	5.5	3.8		4
Gaster length (GL)	4.2	6.1	5.6	5.3	6.5	4.7	3.9	

Intercaste morphology may give insights about the developmental mechanism of caste differentiation. Caste-specific organ growth is generated when an organ primordium responds to developmental signals, such as juvenile hormone (JH), ecdysteroids and insulin-like growth factor (Nijhout, 1994, 1999, 2003; Nijhout and Wheeler, 1982; Hartfelder, 1987; Hartfelder and Emlen, 2005; Wheeler et al., 2006). Usually, these signals are over or below the caste-determining threshold. However, during intercaste development, signals are thought to be at intermediate levels, and as a result, organs can grow to different degrees (Miura et al., 2003; Wheeler et al., 2006). One explanation of the hierarchical pattern is the variation in thresholds of organ primordial responses to caste-determining signals. Intermediate levels of signal may induce traits that have low-response threshold (e.g. ocelli), whereas it fails to stimulate high-threshold traits (wings or thorax segmentation), thus resulting in the limited expression of queen traits in intercastes. In the laboratory, most intercastes of *T. nylanderi* were produced in large colonies (>100 workers) that had just yielded new queens (L. Plateaux, unpubl.). Another

explanation of hierarchical expression is the heterochronic nature of queen-trait development. Miyazaki et al. (2010) reported that wing imaginal discs differentiate earlier than eye and gonad discs in the ant *Myrmecina nipponica*. Organ primordia that develop early seem to be less likely affected by perturbations in later stages. Our study does not contradict this view, because wing development was less likely than eye or gaster growth.

Can intercastes be a source of novel caste phenotypes?

In many unrelated ant lineages, a wingless reproductive caste called “ergatoid queens”, characterized by a worker-like thorax and queen-like gaster and reproductive organs, has evolved (Peeters, 2012). Ergatoid queens show striking morphological similarities to various intercastes, and both uncouple traits associated with flight vs. reproduction (Molet et al., 2009, 2012). If the propensity to produce intercastes (i.e. developmental plasticity) is genetically encoded, selection of intercastes is possible. Thus, if an anomalous novel phenotype brings benefits to colonies, it can be selected

and become produced regularly through phenotypic accommodation (West-Eberhard, 2003; Molet et al., 2012). In our study, the pattern of queen-trait expression in intercastes indicated that gaster size can increase without substantial development of thoracic characters. Our ethanol-preserved samples did not allow examination of reproductive organs, but Plateaux (1970, p 415–423) showed that ovary and spermatheca can develop in some intercastes lacking wings and/or flight sclerites in thorax. Conversely, various queen-like intercastes lacked a spermatheca or had the same number of ovarioles as workers. Thus, certain combinations of traits in intercastes can result in cheaper non-flying reproductives, although there is no evidence of an immediate function in *T. nylanderi*. In *T. crassipinus*, larger workers and worker-like intercastes have ovarioles more numerous and longer than normal workers, suggesting a propensity for reproduction (El-Shehaby et al., 2012). A focus on intercastes in more ant species, and developmental comparisons with ergatoid queens, may help to understand the evolutionary significance of these alternative phenotypes.

Acknowledgments We thank members of the Laboratory of Ecology and Genetics, Hokkaido University, for helpful discussions and use of the scanning electronic microscope. Masato Abe and Koji Sakakawa helped with data analysis. Sylvain Londe and Mathieu Molet gave useful comments on earlier versions of this manuscript. YO was supported by JSPS Fellowship for Young Scientists (post-doc. in Paris), and by Kakenhi #24870006.

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