Insect. Soc. 50 (2003) 390–397
0020-1812/03/040390-08
DOI 10.1007/s00040-003-0695-z

Insectes Sociaux

Research article

Shift in the behaviours regulating monogyny is associated with high genetic differentiation in the queenless ant *Diacamma ceylonense*

E. Baudry [1,2], C. Peeters, L. Brazier, M. Veuille [1,2] and C. Doums [1,2]

[1] *Laboratoire de Biologie Intégrative des Populations, E.P.H.E.*
[2] *Laboratoire d'Ecologie, UMR 7625 CNRS, Université Pierre et Marie Curie, 7 quai St Bernard, 75005 Paris, France, e-mail: ebaudry@snv.jussieu.fr*

Received 23 January 2003; revised 23 June 2003; accepted 1 July 2003.

Summary. In the queenless ant genus *Diacamma*, one mated worker (called gamergate) maintains reproductive monopoly in a colony by mutilating newly emerged workers. However, in several populations from south India, referred to as *'nilgiri'*, gamergates do not mutilate their nestmates but monopolize reproduction using dominance interactions . Various lines of evidence indicate that *'nilgiri'* populations are closely related to the neighboring species *D. ceylonense*. To determine whether this important behavioural difference between *'nilgiri'* and *D. ceylonense* is associated with significant genetic differentiation, we have used microsatellite and mitochondrial markers to examine genetic variation within and between *'nilgiri'* and *D. ceylonense*. We found a very high genetic differentiation between the two forms, which suggests a lack of gene flow. There was an unexpected pattern of mitochondrial variation, because all *'nilgiri'* populations show identical or very closely related COII sequences except one population with a very different haplotype. This divergent haplotype is genetically much more distant from the other *'nilgiri'* haplotypes than are *D. ceylonense* haplotypes. This pattern is not observed at the nuclear level, which suggests that introgression of mitochondrial DNA probably occurred in some *'nilgiri'* populations.

Key words: Regulation of reproduction, genetic differentiation, microsatellites, mtDNA introgression, gamergates.

Introduction

Social insects generally exhibit extreme reproductive skew and this is achieved in various ways. In the ant subfamily Ponerinae, several species lack the queen caste altogether (Peeters, 1991). In such ants, workers have retained a functional spermatheca and are able to mate and produce diploid offspring. In colonies of queenless ants, one or more mated worker, called gamergate, monopolizes reproduction using dominance hierarchies based on aggression (Monnin and Peeters, 1999; Monnin and Ratnieks, 2001). However, in the monogynous genus *Diacamma*, the gamergate enforces her monopoly by a unique behavioural mechanism. All workers are born with a pair of innervated dorsal appendages ('gemmae') that apparently release an exocrine signal (Peeters and Billen, 1991; Gronenberg and Peeters, 1993). Only workers that keep their gemmae are able to perform sexual calling and mate with a foreign male. The gamergate mutilates the gemmae of all freshly emerged workers, thus irreversibly preventing them from sexual reproduction. This mode of regulation of monogyny appears to be general in the *Diacamma* genus (Fukumoto et al., 1989; Peeters and Higashi, 1989; Sommer et al., 1993; André et al., 2001; Cuvillier-Hot et al., 2002) with only one known exception. In several Indian populations, referred to as *'nilgiri'*, the gamergates do not mutilate their nestmates and yet retain reproductive monopoly (Peeters et al. 1992, in which *'nilgiri'* is incorrectly called *D. vagans*). These populations offer a unique opportunity to investigate evolutionary shifts in reproductive regulation.

As a first step, we assessed the level of genetic differentiation between these peculiar populations and their closest known relatives. Several lines of evidence suggest that *'nilgiri'* is closely related to *D. ceylonense*, of which the nearest population is about 80 kilometres distant (Fig. 1). (i) *'nilgiri'* workers are morphologically indistinguishable from *D. ceylonense* except that they all retain their gemmae. (ii) A molecular phylogeny of the *Diacamma* genus has shown that *D. ceylonense* is the closest known relative of *'nilgiri'* (http://www.biologie.ens.fr/fr/ecologie/thematiques/socialiteetgenetique/molecularphylogeny/molecularphylogeny.html). (iii) Crossing experiments in the laboratory have demonstrated that *'nilgiri'* and *D. ceylonense* can produce viable F1 hybrids (L. Cournault, unpublished results), though it is not currently known whether these F1 hybrids are fertile. These data indicate that *'nilgiri'* are closely related to *D. ceylonense*

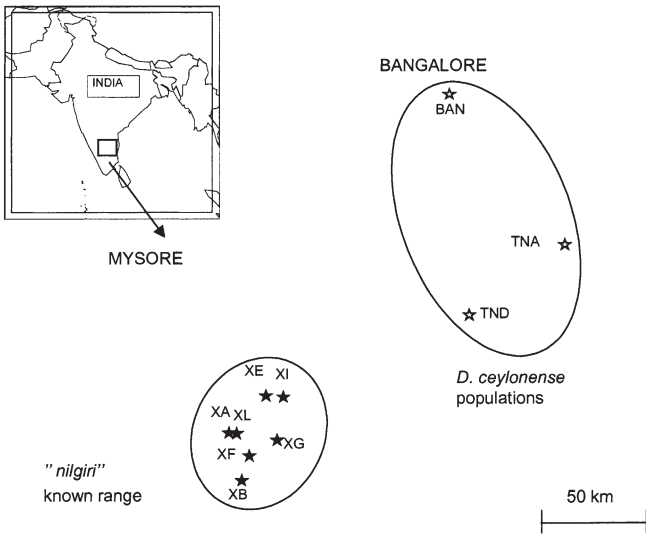

Figure 1. Geographical location of *'nilgiri'* (filled stars) and *D. ceylonense* populations (open stars) in the states of Karnataka and Tamil Nadu. The known range of '*nilgiri*' includes the town of Masinugodi

but we ignore whether they are only atypical populations or a distinct species. To account for this ambiguity, we thereafter refer to *'nilgiri'* and *D. ceylonense* as 'forms'.

To determine whether the crucial difference in behavioural regulation between *'nilgiri'* and *D. ceylonense* is associated with significant genetic differentiation, we examined nuclear and mitochondrial variation for seven populations of *'nilgiri'* and three populations of *D. ceylonense*. We then compared the differentiation observed within *'nilgiri'* populations and within *D. ceylonense* populations to that between the two forms.

Material and methods

Samples

D. ceylonense is distributed in the south east of India (Karnataka and Tamil Nadu states) and in Sri Lanka, while *'nilgiri'* inhabits a very small area (about 10 × 30 km) north of the Nilgiri Hills, on the western edge of the *D. ceylonense* range (C. Peeters, pers. obs.). *'nilgiri'* was sampled at seven localities that cover their entire known range. *D. ceylonense* was sampled at three localities located more than 60 km apart (Fig. 1). In each population, a single worker from 15 to 27 colonies was analysed with seven microsatellite loci (Table 1). Only one individual was sampled per colony to avoid the non-independence of genotypes due to intracolony relatedness. The maximum distance between two colonies of a population was about one kilometre.

Genetic analyses

DNA was extracted from whole workers with QIAgen DNAeasy kit, following the manufacturer protocol (Valencia, CA). Extracted DNA was resuspended in 100 µl elution buffer.

To study nuclear polymorphism, we used 4 microsatellites (DC8, DC11, DC20 and DC52) developed for *Diacamma cyaneiventre* that are polymorphic in *'nilgiri'* or *D. ceylonense* (Doums, 1999) and another 3 loci (DCI-56, DCI-78 and DCI-2122) developed for *D. ceylonense* (Gopinath, 2001). One primer of each pair was 5′-labeled with 6-FAM, NED or VIC derivative of fluoerescein (Applied Biosystems). Each PCR reaction was run in a 10 µl volume containing 1 µl of DNA solution, 100 µM of each dNTP, 0.15 µM of each primer and 0.1 units of Taq polymerase. Loci DC8, DC20, DC52, DCI-56, DCI-78 and DCI-2122 were coamplified. Thermocycle conditions were 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, for a total of 30 cycles. The PCR products were loaded on an ABI Prism 310 (Applied Biosystems). Allele sizes were estimated using the GENESCAN software. For six out of seven loci, we observed shared alleles between *'nilgiri'* and *D. ceylonense* (see Results). To determine whether these shared alleles are truly identical by descent, we sequenced one shared allele per locus in one individual from both *'nilgiri'* and *D. ceylonense*.

Mitochondrial DNA variation was assayed following the amplification of a 731 bp fragment of the Cytochrome Oxidase II gene. The fragment was amplified by PCR using the following primer pair.

CO2-P ATA AAG AAT TTC TTT TAT TA and
CO2-M AAA ATC AAT ACT AAT TAA GT

Each PCR reaction was run in a 50 µl volume containing 1 µl of DNA solution, 200 µM of each dNTP, 0.15 µM of each primer and 1.25 units of Taq polymerase. Thermocycle conditions were 94 °C for 45 s, 47 °C for 45 s, and 72 °C for 1 min, for a total of 30 cycles. Purified template DNA was sequenced with an ABI 310 automatic sequencer (Perkin-Elmer). To determine the importance of within population polymorphism, 15 individuals were first scored in the *'nilgiri'* population XE. Given the very low level of variability that we observed (see Results), only five individuals per population were subsequently scored in all other populations.

Data analysis

The analysis of the microsatellite data was carried out with GENEPOP version 3.3 (Raymond and Rousset, 1995) and Arlequin version 2.00 (Schneider et al., 2000). Hardy-Weinberg equilibrium was tested for the seven loci at each locality using exact probability tests employing a Markov chain to estimate without bias the exact probability value of this test (Guo and Thompson, 1992). To ensure independence of loci, we tested linkage disequilibrium in each population between all pairs of loci. For both Hardy-Weinberg and linkage disequilibrium tests, we use the Bonferroni correction to take multiple tests into account.

The significance of the genetic differentiation between localities was examined by conducting exact tests of allele frequency differentiation with GENEPOP 3.3. The joint probability of differentiation over all microsatellite loci was obtained using Fisher's combined probability test (Sokal and Rolf, 1995). Additionally, we have calculated both F_{ST} (Weir and Cockerham 1984) and R_{ST} statistics (Slatkin, 1995; Rousset, 1996). F_{ST} statistics are based on probabilities of identity between alleles and have been developed for loci evolving under an Infinite Allele Model (IAM). In contrast, R_{ST} statistics are based on size differences between alleles and have been developed for the estimation of genetic distances and population differentiation under a Stepwise Mutation Model (SMM). Because microsatellites are thought to evolve predominantly under SMM (Rienzo et al., 1998; Weber and Wong, 1993; Xu et al., 2000), R_{ST} statistics could be thought as better suited to the analysis of microsatellite data. However, a recent study (Balloux and Goudet, 2002) has shown that even under a strict SMM none of the two statistics is best overall, the best estimator being function of the level of gene flow. We have thus calculated both statistics. To determine whether population and form have an influence on genetic variability, we have performed a hierarchical estimation of *F* and *R* statistics with a two-level hierarchical AMOVA (Evans, 1995) using the software Arlequin (Schneider et al., 2000).

We have tested whether Slatkin's isolation-by-distance model (Slatkin, 1983) of increased genetic distance with increased geographic distance between populations is appropriate for populations of *'nilgiri'*. The significance of the Pearson correlation coefficient between genetic differentiation and geographic distance was assessed with a Mantel test using GENEPOP 3.3.

Table 1. Geographical location of populations and genetic diversity of mitochondrial and microsatellite loci, with Ncol the number of sampled colonies, mtDNA haplotype the mitochondrial haplotypes observed in five individuals, $\overline{Na}$ the mean number of microsatellite allele ± standard deviation and $\overline{He}$ the mean heterozygosity ± standard deviation

Population	Location	Ncol	mtDNA haplotype	$\overline{Na}$ ± s.d.	$\overline{He}$ ± s.d.
'*nilgiri*'					
XA	11°42′12.6″N 76°38′28.8″E	23	H1, H5	2.6 ± 1.3	0.35 ± 0.21
XB	11°34′32.7″N 76°38′48.2″E	15	H7, H8	3.0 ± 1.9	0.41 ± 0.29
XE	11°47′22.7″N 76°45′53.8″E	18	H1, H6	2.7 ± 1.4	0.46 ± 0.25
XF	11°39′03.3″N 76°39′07.9″E	27	H2	3.7 ± 2.0	0.46 ± 0.22
XG	11°39′25.4″N 76°42′41.0″E	15	H3	5.1 ± 2.7	0.65 ± 0.19
XI	11°46′58.2″N 76°47′44.3″E	21	H1	3.3 ± 1.6	0.34 ± 0.18
XL	11°42′08.0″N 76°38′59.0″E	20	H4	2.3 ± 1.0	0.44 ± 0.29
D. ceylonense					
BAN	13°00′00.0″N 77°32′00.0″E	22	H9	3.7 ± 1.9	0.48 ± 0.26
TNA	12°36′52.0″N 77°52′51.0″E	21	H10	6.3 ± 4.1	0.70 ± 0.27
TND	12°04′13.0″N 77°29′15.4″E	19	H11	3.5 ± 1.0	0.51 ± 0.12

The genetic relationships between the observed mitochondrial haplotypes were investigated by constructing a minimum spanning tree of the haplotypes with ARLEQUIN 2.00 (Schneider et al., 2000). The tree was constructed using Kimura two parameters distance (Kimura, 1980). For comparison between the mtDNA and nuclear data, relationships between *'nilgiri'* populations were also established from microsatellite genotypes with the neighbour-joining algorithm using Cavalli-Sforza and Edwards' chord distance (1967), based on allele identity and Goldstein et al. distance (1995), based on allele size.

Results

Within-population genetic diversity

For the seven *'nilgiri'* populations, the number of microsatellite alleles per locus varied between 4 (DC8 and DC11) and 15 (DCI-56). These values ranged from 5 (DCI-78) to 25 (DC52) for the three *D. ceylonense* populations. Observed heterozygosities within populations over all loci range from 0.34 to 0.65 among *'nilgiri'* populations and from 0.48 to 0.70 among *D. ceylonense* (Table 1). Among 210 linkage disequilibrium tests, only seven were significant at the 0.05 level and none were significant after a Bonferroni correction. All the loci can therefore be considered as genetically independent. Only one of the 70 Hardy-Weinberg tests was significant at the 0.05 level and none were significant after a Bonferroni correction. We have thus considered all ten populations to be at Hardy-Weinberg equilibrium.

We observed a very low level of within-population variation at the mitochondrial COII locus. In *D. ceylonense*, a single haplotype is present per population (Table 1). Only one haplotype is observed in four out of seven *'nilgiri'* populations. Three populations, XA, XB and XE exhibit two haplotypes. In all three cases, the second haplotype is found only once and it differs from the more frequent one by one base pair only.

Genetic differentiation between populations and between forms

Exact tests of genic differentiation computed for all pairs of populations, within and between forms, were highly significant at each microsatellite locus, as well as over all loci ($p < 10^{-5}$). In agreement with this important differentiation, we observed high values of F_{ST} and R_{ST} both between forms and within forms (Table 2). Within *'nilgiri',* both statistics indicate a similar level of genetic differentiation. F_{ST} values for population pairs range from 0.001 to 0.374 with a value of 0.213 over all populations. R_{ST} range from 0.008 to 0.461 with a value of 0.259 over all populations. F_{ST} and R_{ST} values for a given pair of *'nilgiri'* populations are not significantly different (Wilcoxon signed rank test, $p = 0.17$). Slatkin's isolation-by-distance model (1983) of increased genetic distance with increased geographic distance between populations was not verified among populations of *'nilgiri'*. Indeed, a Mantel test detected no significant correlation between geographic and genetic distance estimated through F ($p = 0.33$) or R ($p = 0.18$) statistics.

Between the two forms, F (allele identity) and R (allele size) statistics differ markedly (Table 2). F_{ST} values between a *'nilgiri'* and a *D. ceylonense* population have an average of 0.358 (min. 0.146, max. 0.484) while R_{ST} values have an average twice higher of 0.734 (min. 0.529, max. 0.897). A Wilcoxon signed rank test showed that *Rst* values between

Table 2. Pairwise, multilocus estimates of F_{ST} are shown below the diagonal and R_{ST} above. Comparisons between '*nilgiri*' and *D. ceylonense* are in bold

	'*nilgiri*'							D. ceylonense		
	XA	XB	XE	XF	XG	XI	XL	BAN	TNA	TND
XA		0.293	0.134	0.107	0.392	0.406	0.008	**0.737**	**0.722**	**0.914**
XB	0.140		0.250	0.133	0.227	0.217	0.336	**0.587**	**0.576**	**0.826**
XE	0.146	0.242		0.037	0.160	0.362	0.162	**0.680**	**0.665**	**0.881**
XF	0.228	0.275	0.169		0.175	0.238	0.137	**0.684**	**0.682**	**0.854**
XG	0.131	0.177	0.131	0.113		0.395	0.410	**0.592**	**0.595**	**0.813**
XI	0.253	0.344	0.266	0.374	0.253		0.461	**0.578**	**0.567**	**0.783**
XL	0.001	0.150	0.169	0.237	0.130	0.309		**0.742**	**0.723**	**0.925**
BAN	**0.528**	**0.498**	**0.529**	**0.519**	**0.381**	**0.505**	**0.540**		0.005	0.308
TNA	**0.370**	**0.347**	**0.363**	**0.392**	**0.221**	**0.374**	**0.380**	0.113		0.244
TND	**0.461**	**0.416**	**0.454**	**0.449**	**0.298**	**0.501**	**0.462**	0.360	0.217	

forms are significantly higher than the corresponding F_{ST} values ($p < 10^{-4}$). However, if the values of the two statistics differ markedly between the two forms, they are both much higher than the values observed within *'nilgiri'*. The higher values of between forms differentiation obtained with R_{ST} compared to F_{ST} can largely be explained by the very different allelic distribution in *'nilgiri'* and *D. ceylonense* at three loci. For DC11, DC52 and DCI-56, in *D. ceylonense* populations we observe many longer alleles that are absent or very rare in *'nilgiri'* populations (Fig. 2). This pattern is extreme at DC11, for which there is no shared allele between the two forms. In contrast, four loci (DC8, DC20, DCI-78 and DCI-2122) show relatively similar allelic distribution between the two forms, with many shared alleles. To determine whether these shared alleles are identical by descent or are due to size homoplasy, we sequenced one shared allele per locus in one *'nilgiri'* and one *D. ceylonense* individual. We found identical sequences between the two forms, suggesting that the shared alleles are truly identical by descent.

The hierarchical analysis of microsatellite variation (Table 3) confirms the above results by clearly showing the contrasting pattern between allele identity (*F*) versus allele size (*R*) statistics between the forms. The fixation indices calculated from allele identity variation and allele size variation are close within forms (0.22 versus 0.19) but between forms values are much higher when *R* statistics are used compared to *F* statistics (0.73 *versus* 0.30). All fixation indices are highly significant ($p < 10^{-5}$). Likewise, the percentage of variation explained between forms is much higher when allele size variation is used compared to allele identity variation (73.1 versus 29.8%).

The mitochondrial haplotypes found in *'nilgiri'* and *D. ceylonense* populations and the estimated phylogenetic relationships between these haplotypes are shown in Fig. 3a. These relationships show a surprising pattern. Three out of seven *'nilgiri'* populations (XA, XE and XI) show the same haplotype H1. The mtDNA haplotypes of three other populations (XF, XG and XL) differ respectively by only four, two and one substitutions from H1. However the XB population shows two closely related haplotypes (H7 and H8) that are extremely divergent from H1. In fact, the genetic distance between H1 and H7-H8 is much higher than the distance between H1 and the *D. ceylonense* haplotypes, H9, H10 and H11.

To compare this unexpected mtDNA pattern with the nuclear relationships between populations, we used a neighbour-joining tree of populations based on microsatellite data. Fig. 3b shows the tree constructed with the chord distance of Cavalli-Sforza and Edwards' (1967), based on allele identity. A similar topology was obtained with Goldstein's distance (1995), which is based on allele size differences (not shown). The tree reveals two clearly distinct clusters that correspond to *'nilgiri'* and *D. ceylonense* populations (Fig. 3b). Population XB of *'nilgiri'* with a very divergent mtDNA haplotype thus falls well within the *'nilgiri'* clade based on nuclear data.

To confirm the position of XB within the *'nilgiri'* clade, we assayed nuclear polymorphism using two rDNA internal transcribed spacers. A 500 bp fragment of both ITS1 and ITS2 was PCR amplified and sequenced using universal primers (Campbell et al., 1993; Sappal et al., 1995). We obtained identical sequences at the two loci for four *'nilgiri'* individuals (two from XA and two from XB) and two *D. ceylonense* individuals from TNA. This lack of differences between *'nilgiri'* and *D. ceylonense* does not allow to infer the position of XB within the *'nilgiri'* clade but it indicates that the two forms diverged very recently.

Discussion

Within-form genetic structuring

We observed a high level of nuclear genetic differentiation between the populations of each form. Similar studies made in other ant species also detected significant genetic differentiation between populations but the F_{ST} values were lower. For example, the F_{ST} values between populations were respectively 0.11, 0.17 and 0.19 in *Formica cinerea*, *Gnamptogenys striatula* and *Camponotus floridanus* (Gadau et al., 1996; Giraud et al., 2000; Goropashnaya et al., 2001). This is especially remarkable given that the geographical distances between populations in the above studies were much higher than those separating '*nilgiri'* populations. In contrast, a recent study of *Diacamma cyaneiventre* by Doums et al.

Table 3. Hierarchical analysis of molecular variance using either allele identity or the sum of squared allele size difference. The significance of the fixation indices was assayed using the non-parametric approach described in Excoffier et al. (1992), consisting in permuting 1000 times individuals or populations among populations or forms, depending on the hierarchical level tested. All fixation indices are highly significant and have a probability inferior to 10^{-5}

	Allele identity		Squared allele size difference	
	Percent of variation	F_{ST}	Percent of variation	R_{ST}
Between forms	29.8%	0.30	73.1%	0.73
Within form, between populations	15.1%	0.22	5.1%	0.19

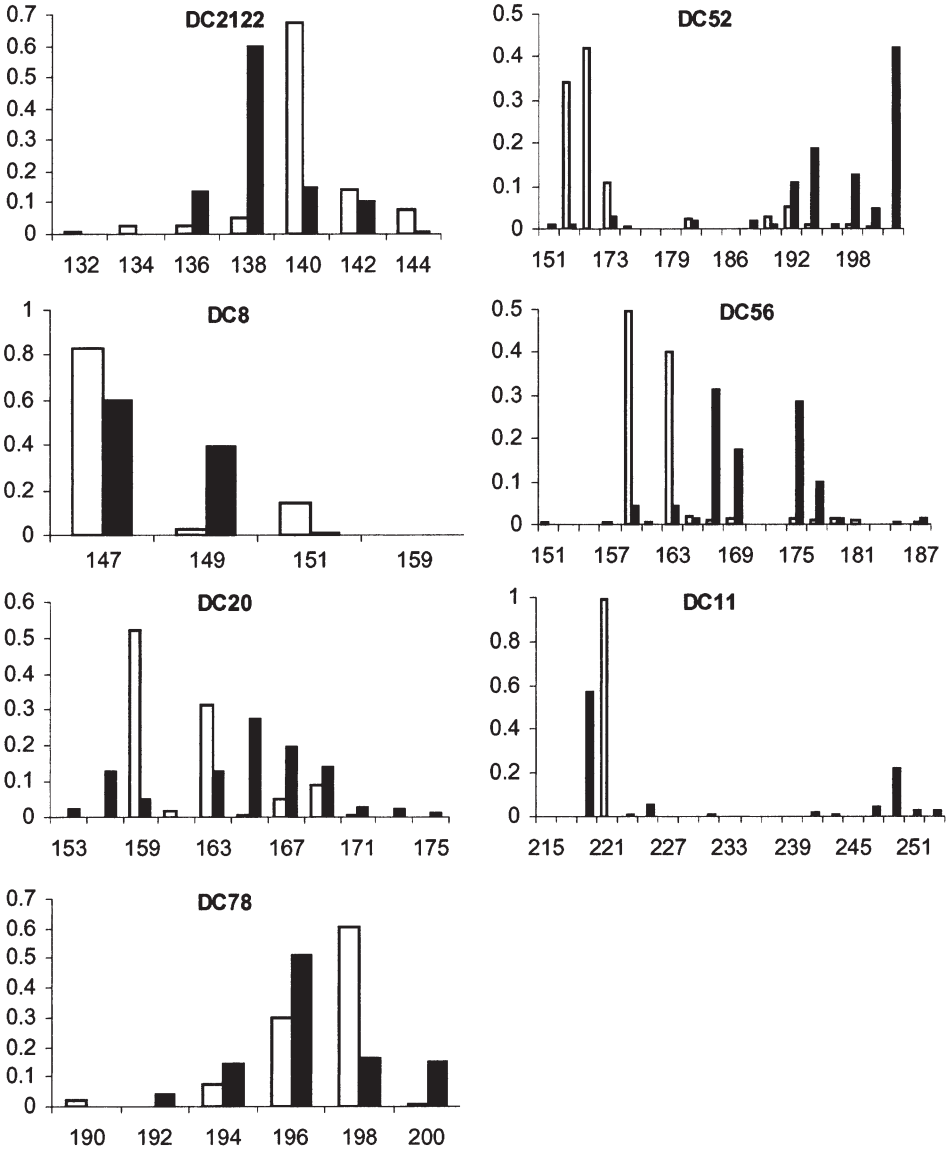

Figure 2. Allele distribution at seven microsatellite loci in *'nilgiri'* (open bars) and *D. ceylonense* (solid bars). Allele size is indicated as number of base pairs

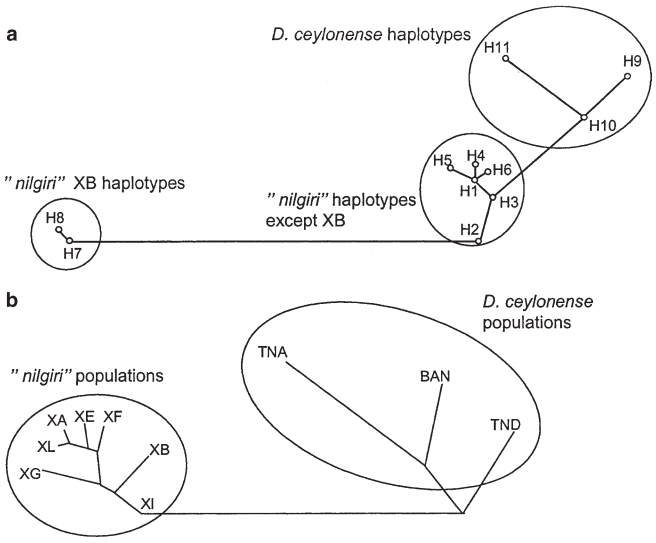

Figure 3. Comparison of mitochondrial and nuclear relationships. a: Minimum spanning tree reflecting the relationships between mtDNA haplotypes. The tree was constructed using Kimura two parameters distance (Kimura, 1980). b: Neighbour-joining tree of populations obtained from microsatellite data using Cavalli-Sforza and Edwards' chord distance (1967), based on allele identity

(2002) showed a level of structure between populations similar to that in our study. This suggests that genetic differentiation between populations is usually large in the *Diacamma* genus, although more species need to be studied to confirm this. This high differentiation is most likely the consequence of restricted dispersal. In the *Diacamma* genus, new colonies are founded by a wingless gamergate helped by a group of workers. Female dispersal is therefore restricted to ant 'walking distance'. There is currently little data on male dispersal in *Diacamma* but our results suggest that it is limited. Additionally, genetic differentiation in *'nilgiri'* and *D. ceylonense* could be enhanced by high levels of genetic drift caused by low effective population size. Indeed, all the populations that we sampled are composed of a limited number of colonies, probably a few hundreds. This is in agreement with the relatively low level of intrapopulation genetic variability observed both in *D. ceylonense* and especially in *'nilgiri'* (mean microsatellite heterozygosity of 0.44).

Between forms genetic structuring

We have used both F and R estimates to measure genetic differentiation. Interestingly, if the two types of estimators produce similar values within the forms, R_{ST} values are much higher than F_{ST} values between the forms. This is not unexpected given the high level of differentiation found between the two forms, as R_{ST} is expected to better reflect true differentiation in highly structured populations because the effect of mutation becomes more important than migration (Balloux et al., 2000). This tendency has been confirmed in several empirical studies (cursory review in Lugon-Moulin et al., 1999).

Whatever the estimator used, our results show a very high genetic differentiation between *'nilgiri'* and *D. ceylonense.* The absence of shared alleles at one microsatellite locus and their quasi-absence at two other loci suggest that gene flow is very restricted or even nonexistent between the two forms. Moreover, the very high fixation index between forms (0.73) has to our knowledge never been reported within a species. It would thus be tempting to speculate that the two forms are in reality two distinct species. However, genetic differentiation per se does not allow to conclude about species status. Reproductive isolation is only loosely correlated with genetic distance (Coyne and Orr, 1989, 1998). Therefore, especially when natural or sexual selection is involved, differentiation at neutral loci does not always reflect differentiation at loci governing reproductive isolation (Wu, 2001). Crossing experiments between the two forms and mating preference tests will be necessary to definitely ascertain the species status of *'nilgiri'*.

Mitochondrial variation

We observed an unexpected pattern of mtDNA: while most *'nilgiri'* populations show identical or very closely related mitochondrial haplotypes, the XB population shows a different haplotype that is genetically much more distant from the other *'nilgiri'* haplotypes than are the *D. ceylonense* haplotypes. In contrast, at the nuclear level, this XB population falls well within the *'nilgiri'* clade. Two hypotheses can be proposed to explain this surprising pattern. First, reconstructed relationships between genes can differ from the history of species (Nichols, 2001). The topology of the mtDNA trees of *'nilgiri'* and *D. ceylonense* populations is the result of coalescence events, which are highly stochastic (Hudson, 1990). We could therefore suppose that the observed mtDNA pattern does not reflect any special event but rather the randomness of coalescence events. However, given the very high divergence of the XB haplotype, this hypothesis seems relatively unlikely. Alternatively, we can suppose that a foreign mtDNA introgressed into *'nilgiri'* populations. Several examples of anomalous mtDNA pattern most probably caused by interspecific introgression are known in ants (Shoemaker et al., 2000) and *Drosophila* (Lachaise et al., 2000; Rousset and Solignac, 1995). In our case, the postulated introgression could have occurred in two ways. A first possibility is that the haplotypes from all populations except XB represent the original *'nilgiri'* mtDNA. In such a case, the mtDNA from XB would result from introgression. We have checked that the XB haplotype is not close to any currently known *Diacamma* haplotypes (Veuille, Brusadelli, Brazier and Peeters, unpublished data), so there is no obvious candidate for the origin of the introgressed DNA. Alternatively, if we suppose that the divergent XB haplotype represents the original *'nilgiri'* mtDNA, then it is the haplotypes from the other *'nilgiri'* populations that originated by introgression, most probably from *D. ceylonense* because their haplotypes are very closely related.

The shift away from mutilation in the regulation of monogyny in *'nilgiri'* appears to have resulted in a high

genetic differentiation from *D. ceylonense,* both at the mitochondrial and nuclear levels. Since regulation based on dominance is reversible, this shift could also influence gene flow within *'nilgiri',* because it is possible that matings with foreign males occur more often. In *D. ceylonense*, as in other *Diacamma* species, all workers are mutilated and therefore unable to mate. On the contrary, all *'nilgiri'* workers retain their gemmae and can potentially reproduce sexually. This could lead to a higher frequency of gamergate replacement in *'nilgiri'* and thus to a higher level of gene flow between populations. It would therefore be interesting to study whether the populations of *'nilgiri'* show a lower genetic differentiation than the populations of *D. ceylonense* at a similar spatial scale.

Acknowledgements

We thank Raghavendra Gadagkar and Ashok Gopinath for valuable scientific discussions. We thank Sebastien Baratte and the Bangalore team for help in sample collection. We thank one anonymous reviewer for helpful comments on the manuscript . Our research on indian *Diacamma* is part of a collaborative project with Raghavendra Gadagkar (Indian Institute of Science, Bangalore) and is supported by the CNRS ('PICS').

References

André, J.-B., C. Peeters and C. Doums, 2001. Serial polygyny and colony genetic structure in the monogynous queenless ant *Diacamma cyaneiventre*. *Behav. Ecol. Sociobiol. 50*: 72–80.

Balloux, F., H. Brunner, N. Lugon-Moulin, J. Hausser and J. Goudet, 2000. Microsatellites can be misleading: an empirical and simulation study. *Evolution 54*: 1414–1422.

Balloux, F. and J. Goudet, 2002. Statistical properties of population differentiation estimators under stepwise mutation in a finite island model. *Mol. Ecol. 11*: 771–783.

Campbell, B.C., J.D. Steffen-Campbell and J.H. Werren, 1993. Phylogeny of the *Nasonia* species complex (Hymenoptera: Pteromalidae) inferred from an internal transcribed spacer (ITS2) and 28S rDNA sequences. *Insect. Mol. Biol. 2*: 225–237.

Cavalli-Sforza, L. and A. Edwards, 1967. Phylogenetic analysis: models and estimation procedures. *Am. J. of Hum. Genet. 19*: 233–257.

Coyne, J. A., and H.A. Orr, 1989. Patterns of speciation in *Drosophila*. *Evolution 43*: 362–381.

Coyne, J.A. and H.A. Orr, 1998. The evolutionary genetics of speciation. *Phil. Trans. R. Soc. Lond. B Biol. Sci. 353*: 287–305.

Cuvillier-Hot, V., R. Gadagkar, C. Peeters and M. Cobb, 2002. Regulation of reproduction in a queenless ant: aggression, pheromones and reduction in conflict. *Proc. R. Soc. Lond. B 269*: 1295–1300.

Doums, C., 1999. Characterization of microsatellite loci in the queenless Ponerine ant *Diacamma cyaneiventre*. *Mol Ecol 8*: 1957–1959.

Doums, C., H. Cabrera and C. Peeters, 2002. Population genetic structure and male-biased dispersal in the queenless ant *Diacamma cyaneiventre*. *Mol. Ecol. 11*: 2251–2264.

Evans, J.D., 1995. Relatedness threshold for the production of female sexuals in colonies of a polygynous ant, *Myrmica tahoensis*, as revealed by microsatellite DNA analysis. *Proc. Natl. Acad. Sci. USA 92*: 6514–6517.

Excoffier, L., P. Smouse and J. Quattro, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics 131*: 479–491.

Fukumoto, Y., T. Abe and A. Taki, 1989. A novel form of colony organization in the 'queenless' ant *Diacamma rugosum. Physiol. Ecol. Jpn 26*: 55–62.

Gadau, J., J. Heinze, B. Hölldobler and M. Schmid, 1996. Population and colony structure of the carpenter ant *Camponotus floridanus. Mol. Ecol. 5*: 785–792.

Giraud, T., R. Blatrix, C. Poteaux, M. Solignac and P. Jaisson, 2000. Population structure and mating biology of the polygynous ponerine ant *Gnamptogenys striatula* in Brazil. *Mol. Ecol. 9*: 1835–1841.

Goldstein, D., A. Ruiz Linares, L. Cavalli-Sforza and M. Feldman, 1995. An evaluation of genetic distances for use with microsatellite loci. *Genetics 139*: 463–471.

Gopinath, A., 2001. Identification of polymorphic loci in the queenless, ponerine ant *Diacamma ceylonense*. *Mol. Ecol. Notes 1*: 126–127.

Goropashnaya, A., P. Seppa and P. Pamilo, 2001. Social and genetic characteristics of geographically isolated populations in the ant *Formica cinerea*. *Mol. Ecol. 10*: 2807–2818.

Gronenberg, W. and C. Peeters, 1993. Central projections of the sensory hairs on the gemma of the ant *Diacamma*: substrate for behavioral modulation? *Cell Tissue Res. 273*: 401–415.

Guo, S. and E. Thompson, 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics 48*: 361–372.

Hudson, R.R., 1990. Gene genealogies and the coalescent process. In: *Oxford Surveys in Evolutionary Biology* (D.F.A.J. Antonovics, Ed.), pp. 1–44, Oxford University Press, Oxford.

Kimura, M., 1980. A simple method for estimating evolutionary rates of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol. 16*: 111–120.

Lachaise, D., M. Harry, M. Solignac, F. Lemeunier, V. Benassi and M.L. Cariou, 2000. Evolutionary novelties in islands: *Drosophila santomea*, a new *melanogaster* sister species from Sao Tome. *Proc. R. Soc. Lond. B. 267*: 1487–1495.

Lugon-Moulin, N., H. Brunner, A. Wyttenbach, J. Hausser and J. Goudet, 1999. Hierarchical analyses of genetic differentiation in a hybrid zone of *Sorex araneus*. *Mol. Ecol. 8*: 419–431.

Monnin, T. and C. Peeters, 1999. Dominance hierarchy and reproductive conflicts among subordinates in a monogynous queenless ant. *Behav. Ecol. 10*: 323–333.

Monnin, T. and F. Ratnieks, 2001. Policing in queenless ponerine ants. *Behav. Ecol. Sociobiol. 50,* 97–108.

Nichols, R., 2001. Gene trees and species trees are not the same. *Trends Ecol. Evol. 16*: 358–364.

Peeters, C., 1991. The occurrence of sexual reproduction among ant workers. *Biol. J. Linn. Soc. 44*: 141–152.

Peeters, C. and J. Billen, 1991. A novel exocrine gland inside the thoracic appendages ('gemmae') of the queenless ant *Diacamma australe*. *Experientia 47*: 229–231.

Peeters, C. and S. Higashi, 1989. Reproductive dominance controlled by mutilation in the queenless ant *Diacamma australe*. *Naturwissenschaften 76*: 177–180.

Peeters, C., J. Billen and B. Hölldobler, 1992. Alternative dominance mechanism regulating monogyny in the queenless ant genus *Diacamma*. *Naturwissenshaften 79*: 572–573

Raymond, M. and F. Rousset, 1995. GENEPOP (version 1.2): A population genetics software for exact test and ecumenicism. *J. Hered. 86*: 248–249.

Rienzo, A.D., P. Donnelly, C. Toomajian, B. Sisk, A. Hill, M. L. Petzl-Erler, G.K. Haines and D.H. Barch, 1998. Heterogeneity of microsatellite mutations within and between loci, and implications for human demographic histories. *Genetics 148*: 1269–1284.

Rousset, F., 1996. Equilibrium values of measures of population subdivision for stepwise mutation processes. *Genetics 142*: 1357–1362.

Rousset, F. and M. Solignac, 1995. Evolution of single and double *Wolbachia* symbioses during speciation in the *Drosophila simulans* complex. *Proc. Natl. Acad. Sci. USA 92*: 6389–6393.

Sappal, N.P., R.S. Jeng, M. Hubbes and F. Liu, 1995. Restriction fragment length polymorphisms in polymerase chain reaction amplified ribosomal DNAs of three *Trichogramma* (Hymenoptera: Trichogrammatidae) species. *Genome 38*: 419–425.

Schneider, S., D. Roessli and L. Excoffier, 2000. Arlequin: A software for population genetics data analysis, Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Shoemaker, D.D., K.G. Ross, L. Keller, E.L. Vargo and J.H. Werren, 2000. *Wolbachia* infections in native and introduced populations of fire ants (*Solenopsis* spp.). *Insect Mol. Biol. 9*: 661–673.

Slatkin, M., 1983. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution 47*: 264–279.

Slatkin, M., 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics 139*: 457–462.

Sokal, R.R. and F.J. Rolf, 1995. *Biometry*, Freeman & Company, New York.

Sommer, K., B. Hölldobler and H. Rembold, 1993. Behavioral and physiological aspects of reproductive control in a *Diacamma* species from Malaysia (Formicidae, Ponerinae). *Ethology 94*: 162–170.

Weber, J. and C. Wong, 1993. Mutation of human short tandem repeats. *Hum. Mol. Genet. 2*: 1123–1128.

Weir, B. and C. Cockerham, 1984. Estimating F-statistics for the analysis of population structure. *Evolution 38*: 1358–1370.

Wu, C., 2001. The genic view of the process of speciation. *J. Evol. Biol. 14*: 851–865.

Xu, X., M. Peng and Z. Fang, 2000. The direction of microsatellite mutations is dependent upon allele length. *Nat. Genet. 24*: 396–399.