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# Population genetic structure and male-biased dispersal in the queenless ant *Diacamma cyaneiventre*

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**Abstract**

**In this study we investigated the population genetic structure of the queenless ant *Diacamma cyaneiventre*. This species, lacking winged queens, is likely to have a restricted female dispersal. We used both mitochondrial and microsatellite markers to assess the consequence of such restricted female dispersal at three geographical scales: within a given locality (< 1 km), between localities within a given region (< 10 km) and between regions (> 36 km). Within a locality, a strong population structure was observed for mitochondrial DNA (mtDNA) whereas weak or nonexistent population genetic structure was observed for the microsatellites (around 5% of the value for mtDNA). Male gene flow was estimated to be about 20–30 times higher than female gene flow at this scale. At a larger spatial scale, very strong genetic differentiation for both markers was observed between localities — even within a single region. Female dispersal is nonexistent at these scales and male dispersal is very restricted, especially between regions. The phylogeographical structure of the mtDNA haplotypes as well as the very low genetic diversity of mtDNA within localities indicate that new sites are colonized by a single migration event from adjacent localities, followed by successive colony fissions. These patterns of genetic variability and differentiation agree with what is theoretically expected when colonization events are kin-structured and when, following colonization, dispersion is mainly performed by males.**

*Keywords*: *Diacamma cyaneiventre*, gamergate, hierarchical genetic structure, microsatellites, mtDNA, population viscosity

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## Introduction

Dispersal is a fundamental life history trait affecting gene flow and therefore shaping the distribution of genetic variability within and between populations (Waser & Strobeck 1998). In social insects, especially in ants where workers are wingless, patterns of dispersal are linked tightly to the mode of colony foundation. Colony foundation can be independent when the queen(s) founds the colony alone, or dependent when the queen(s) is helped by a group of workers. Female dispersal is restricted to 'walking distance' under dependent colony foundation whereas independent colony foundation is often associated with mating in flight, providing the potential for dispersal to take place over longer distances (Peeters & Ito 2001).

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In some polygynous ant species, in which dependent colony foundation predominates, restricted female gene flow within populations (at the scale of a few hundred metres) was detected using mitochondrial DNA (Stille & Stille 1993; Ross *et al*. 1997; Tay *et al*. 1997; Goodisman & Ross 1998; Liautard & Keller 2001). On the other hand, in monogynous forms of *Solenopsis invicta* in which the queen founds the colony alone, genetic differentiation for mtDNA was detected at the scale of few kilometres but not at the scale of few hundred metres within a given population (Ross *et al*. 1997).

Restricted female dispersal can induce some population viscosity for nuclear genes, i.e. an increase in genetic similarity between neighbouring colonies, if male dispersal is also restricted. A knowledge of local population genetic structure is important when analysing breeding system and within-colony genetic structure (Ross 2001). By increasing the genetic relatedness between interacting

individuals, population viscosity can also affect the evolution of social behaviours (Kelly 1992; Queller 1992). Many studies on ants have investigated the extent of population viscosity, and tend to indicate that polygyny is generally associated with population viscosity (Pamilo 1983; Crozier *et al*. 1984; Crozier & Pamilo 1986; Seppä & Pamilo 1995; Chapuisat *et al*. 1997; Beye *et al*. 1998; Giraud *et al*. 2000; Tsutsui & Case 2001), whereas no or very low population viscosity is detected in monogynous species or forms (Sundström 1993; Seppä & Pamilo 1995; Ross *et al*. 1997). Population viscosity is expected in polygynous species but not in monogynous species for two reasons. First, polygyny is often associated with dependent colony foundation and monogyny with independent colony foundation (Bourke & Franks 1995; Keller 1991). Second, movements between nests produced by budding has been found in polygynous species but is extremely rare in monogynous species (e.g. Chapuisat *et al*. 1997). Such exchange of workers can decrease in intensity with increasing geographical distance between nests and lead to some population viscosity. In this case, population viscosity does not necessarily reflect limited sexual female gene flow, as the pattern of microgeographical structure can disappear when nests from different colonies are studied (e.g. Chapuisat *et al*. 1997).

Queenless ants of the genus *Diacamma* provide an original model for investigating the consequences of restricted female dispersal on population genetic structure. In queenless species, female dispersal is limited to ant walking distance. These species have a dependent mode of colony foundation and are monogynous with a single nest per colony (Peeters *et al*. 1992). Males are winged and have the potential to disperse genes over longer distances than females. Such potential male-biased dispersal should leave some trace in the population genetic structure when biparentally and maternally inherited markers are compared (Ennos 1994). The consequence of limited female dispersal on the population genetic structure of markers with constrasting modes of inheritance has rarely been investigated in ants (but see Ross *et al*. 1997; 1999).

In this study, we used variation in mtDNA and in eight microsatellite loci to investigate: (i) the extent of microgeographical genetic structure in one locality of the queenless ant *D. cyaneiventre* found in southern India. In this species, the nests extend deep underground (up to 1 m), are the result of an elaborate construction effort and colonies do not emigrate frequently (C. Doums & C. Peeters, unpublished data). *D. cyaneiventre* therefore provides a good model for investigating population viscosity. (ii) The consequence of restricted female dispersal at larger spatial scales, more specifically at two hierarchical scales, i.e. between localities within a region (separated by few kilometres) and between regions (from 36 to 188 km apart). If male gene flow is also restricted, strong genetic differentiation is expected, especially at a larger spatial scale.

## Materials and methods

### *Samples*

*Diacamma cyaneiventre* is a Ponerine ant that inhabits open areas. As far as we know, the distribution area of this species is restricted to the southwest of India. Two hundred and twenty-one colonies of *D. cyaneiventre* were sampled in seven localities distributed in three regions in Karnataka state (Fig. 1). This sampling represents the species' known range. The exact position of each locality, as determined by GPS, is given in Table 1. The number of samples collected was larger in Kottigehara 1 compared to other localities, as this locality had been the subject of a previous study (André *et al*. 2001). Moreover, the topology of this site made it possible to map the colonies. The shape of the sampling area was determined mainly by the dense forest surrounding the site, in which *Diacamma* ants are not found. In *D. cyaneiventre*, the average genetic relatedness between nestmates is 0.75 (André *et al*. 2001). We therefore determined the genotype of only one individual per colony to avoid the nonindependence of genotypes attributable

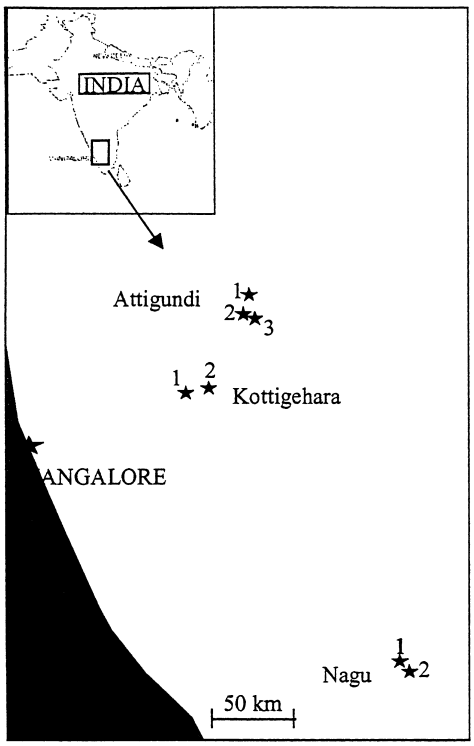


**Fig. 1** Geographical position of *D. cyaneiventre* localities in the south of India. The seven localities sampled, depicted by stars, are distributed in three regions: Attigundi, Kottigehara and Nagu.

**Table 1** Population position and genetic diversity of mitochondrial and microsatellite DNA. *N*, the number of colonies analysed (one individual per colony); $H_a$, the mitochondrial haplotype detected in each site with their accession number in gene bank in parentheses; $H_E^{Nuc}$ and $H_E^{mt}$, the gene diversity for microsatellite (average expected heterozygosity over loci ± SD) and mtDNA (probability that two randomly chosen haplotypes are different ± SD), respectively (Nei 1987)

| Population | Position | *N* | $H_a$ | $H_E^{mt}$ | $H_E^{Nuc}$ ± SD |
|---|---|---|---|---|---|
| Attigundi 1 | 13°30′06,3″ N<br>75°44′22,7″ E | 15 | 1 (AF467546) | 0 | 0.63 ± 0.15 |
| Attigundi 2 | 13°25′01,6″ N<br>75°44′27,4″ E | 12 | 2 (AF467547) | 0 | 0.58 ± 0.28 |
| Attigundi 3 | 13°26′6,5″ N<br>75°44′15,0″ E | 12 | 2 (AF467547) | 0 | 0.54 ± 0.28 |
| Kottigehara 1 | 13°6′57,3″ N<br>75°30′54,2″ E | 101 | 3 (AF467548)<br>4 (AF467549)<br>5 (AF467550) | 0.53 ± 0.23 | 0.64 ± 0.22 |
| Kottigehara 2 | 13°7′16,6″ N<br>75°36′11,4″ E | 20 | 6 (AF467551) | 0 | 0.68 ± 0.21 |
| Nagu 1 | 11°59′39,2″ N<br>76°26′21,3″ E | 24 | 7 (AF467552) | 0 | 0.67 ± 0.22 |
| Nagu 2 | 11°57′21,5″ N<br>76°27′13,5″ E | 28 | 8 (AF467553) | 0 | 0.68 ± 0.19 |

to family structure. Three hierarchical levels of genetic differentiation were therefore investigated: (i) the population viscosity within the locality Kottigehara 1; (ii) the genetic differentiation between localities within a region (a few kilometres apart); and (iii) between regions (from 36 to 188 km apart).

### *Genetic analysis*

DNA was obtained using a rapid extraction procedure (see André *et al*. 2001). Eight microsatellite loci developed for *D. cyaneiventre* (Doums 1999) were used in this study. Polymerase chain reactions (PCR) were conducted following the protocol of Doums (1999), except for the coamplification of loci D19 and D20, for which the amounts of dNTP, $^{33}$PdATP and Taq DNA polymerase were doubled.

Haplotypes of the mtDNA were scored following PCR amplification of a 280-base pair (bp) segment of the COII gene and separation of the products using the single-strand conformation polymorphism analysis (SSCP) technique (Orita *et al*. 1989). This fragment was amplified using the conserved primers (300P 5′-GGTCATCAATGATACTGATC-3′ and 630 M 5′-AATCATAGATTTATACCAAT-3′). Each PCR reaction was run in a 10-μL volume containing 1 μL of DNA solution, 75 μM of each dNTP, 0.025 μci dATP, 0.4 μM of each primer, 1 × taq buffer and 0.25 units of *Taq* polymerase (Quiagen) using a PTC-100 thermal cycler (MJ Research). The thermal cycle profile was as follows: an initial hot-start of 3 min at 94 °C; 30 amplification cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 40 °C and extension for 1 min at 72 °C; and a final extension for 10 min at 72 °C. The amplified products were loaded onto 6% denaturing acrylamide gel. Gels were run at 15 W for 15 h at a constant room temperature of 20 °C and autoradiographed for approximately 24 h. For each haplotype detected, the PCR product of two individuals was sequenced using the Thermo Sequenase Kit (US Biochemical Corp.) after purification using a gel band purification kit (Amersham).

### *Data analysis*

Hardy–Weinberg equilibrium was tested for the eight loci at each locality using exact tests (GENEPOP 3.2; Raymond & Rousset 1995). Fisher's method of combining independent test results (Sokal & Rohlf 1995: 794) was used to determine the overall significance for each locality and each locus. Linkage disequilibrium between pairs of loci was tested for each locality using GENEPOP 3.2.

*Population viscosity*. Three different analyses were conducted to investigate the extent of population viscosity in Kottigehara 1. As the colonies were not distributed continuously throughout the locality, due mainly to environmental heterogeneity (forest, bushes, road and river, Fig. 2), the locality was divided into three demes. Demes 1 and 2 were separated by a river and demes 2 and 3 by dense bushes. However, these three demes were connected by roads (see Fig. 2). In demes 1 and 2 (Fig. 2) the sampling was nearly exhaustive. First, genetic

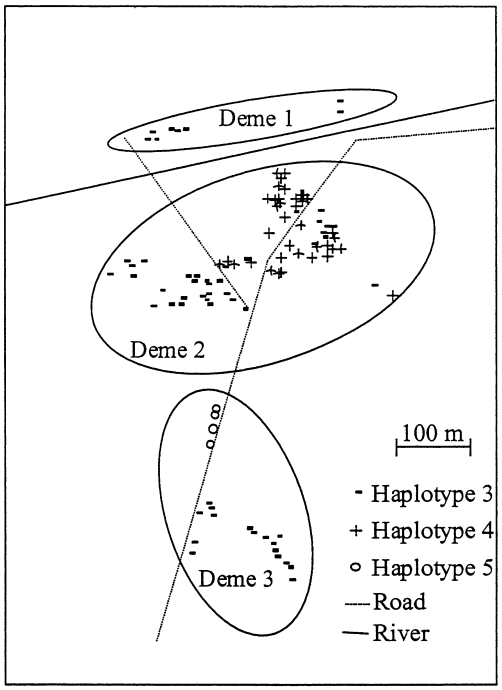


**Fig. 2** Map of *D. cyaneiventre* colonies sampled from Kottigehara 1. Each colony is depicted by a symbol characterizing its mitochondrial haplotype. The three circles characterize the three demes used for estimating the genetic substructuration within this locality.

differentiation among the three demes was analysed using *F*-statistics as described below for both types of makers. Second, the population viscosity was tested by checking whether neighbouring colonies were more closely related than non-neighbouring colonies, using all colonies or only colonies within the largest, deme 2, containing 73 continuously distributed colonies. Neighbouring colonies were defined using a Gabriel graph (Gabriel & Sokal 1969). The average relatedness between neighbouring colonies was calculated using RELATEDNESS 5.0 (Queller & Goodnight 1989). The standard error of the estimate was obtained by jackknifing over individuals and was used to test whether the estimated relatedness was significantly different from 0 using a *t*-test (Sokal & Rohlf 1995). Third, within deme 2, population viscosity was also investigated by plotting the genetic differentiation between colonies against geographical distance (Rousset 2000). The estimator $a_r$ of genetic differentiation between individuals (here colonies), analogous to $F_{ST}/(1 - F_{ST})$, was calculated using GENEPOP 3.3. The significance of the Pearson correlation coefficient between genetic differentiation and geographical distance was assessed with a Mantel test (GENEPOP 3.3, Raymond & Rousset 1995).

*Genetic differentiation between localities.* The significance of the genetic differentiation between localities was examined by conducting exact tests of allele frequency differentiation (GENEPOP 3.2, Raymond & Rousset 1995). The joint probabilities of differentiation over all microsatellite loci were obtained using Fisher's combined probability test (Sokal & Rohlf 1995).

For the microsatellites, we computed both *F*- and *R*-statistics assuming the infinite allele model (Wright 1978) or a stepwise mutation process (Slatkin 1995), respectively. The mutation process will influence estimations of differentiation if the coalescence times of genes between populations are sufficiently long for two or more mutation events to have occurred (Rousset 1996). This may be the case in our study, as female dispersal should be very restricted. Population genetic differentiation was partitioned at two levels; across all localities and between localities within each region (see Fig. 1). Hierarchical *F*-statistics and *R*-statistics were estimated with a two-level hierarchical AMOVA (Excoffier *et al.* 1992) using ARLEQUIN 2.00 (Schneider *et al.* 2000). The same analyses were conducted on either haplotype frequency (equidistant $\phi_{ST}$) or the proportion of nucleotide differences between haplotypes (euclidean $\phi_{ST}$). to estimate equidistant and euclidian $\phi_{ST}$ for the mitochondrial markers (ARLEQUIN 2.00, Schneider *et al.* 2000). *F*-statistics between pair of localities were estimated according to Weir & Cockerham (1984), and *R*-statistics according to Rousset (1996) using FSTAT version 2.8 (Goudet 1995) or GENEPOP 3.3 (Raymond & Rousset 1995). The significance of the Pearson correlation coefficient between genetic differentiation and geographical distance between localities was assessed with a Mantel test (GENEPOP 3.3, Raymond & Rousset 1995).

The genetic relationship between mitochondrial haplotypes was investigated by constructing a minimum spanning tree reflecting the unrooted genealogical relationships between the mtDNA haplotypes using ARLEQUIN 2.00 (Schneider *et al.* 2000) as well as a neighbour-joining tree using MEGA software (Kumar *et al.* 2001). The neighbour-joining tree was rooted at the midpoint of the longest distance between any two haplotypes. The difference between both trees is that haplotypes can be ancestral to other haplotypes in a minimum spanning tree but not in a neighbour-joining tree. For constructing both trees, we used the Jukes and Cantor distance, an appropriate measure for the level of genetic distance detected (Nei & Kumar 2000).

*Male and female gene flow.* Although estimates of *Nm* from $F_{ST}$ cannot be directly translated into a number of migrants moving between populations (Bossart & Prowell 1998; Hedrick 1999; Whitlock & McCauley 1999), these estimates still provide a straightforward method of comparing the relative intensity of male and female gene flow. Under the assumptions of Wright's infinite island model (Wright 1978) at migration-drift equilibrium, genetic differentiation are given by: $F_{ST}^{mt} = 1/(2N_{ef}m_f + 1)$ for maternally inherited

genes with one copy per individual (mtDNA) and a female migration rate of $m_f$, $F_{ST}^{mt} = 1/(4N_e m + 1)$ for biparentally inherited genes (microsatellites) and migration rate $m$ (Hartl & Clark 1997).

For haplodiploid species, $N_e = 9N_{ef}N_{em}/(2N_{ef} + 4N_{em})$ (Hartl & Clark 1997) and under the assumption of dispersal before mating, $m = (2m_f + m_m)/3$ with $m_f$ and $m_m$ being the migration rate of females and males, respectively (Berg *et al*. 1998). This model of dispersal is the most appropriate, as males of *D. cyaneiventre* disperse before mating. *D. cyaneiventre* can be considered a monoandrous species (André *et al*. 2001) and the male loses a part of his abdomen during mating and consequently can mate only once (pers. obs.). We therefore assumed an even effective sex-ratio that is $N_f = N_m = 1/2N$. From these equations and the *F*-statistics estimates of both mitochondrial and microsatellite markers, we estimated male and female gene flow between demes within Kottigehara 1 and between localities at the two spatial scales (within and between regions).

## Results

### *Within-locality genetic diversity*

Allele frequencies and gene diversities (Nei 1987) are given in Appendix I for each locality and each locus. Over all localities, the total number of microsatellite alleles per locus varied between seven (DC5) and 38 (DC18) and the gene diversity between 0.44 (DC20) and 0.90 (DC18). The within-locality genetic diversity for the different localities is given in Table 1.

Eight mitochondrial haplotypes were identified by SSCP. Within a given haplotype, the sequence of the two individuals examined was always identical. Over the entire sample, 29 variable sites were detected out of 263 bp (percentage of polymorphic sites = 11%). The overall nucleotide diversity (Nei 1987: average number of nucleotide differences per site between two sequences) was 3.8% (± 1.9 SD), while the overall gene diversity (Nei 1987: probability that two randomly chosen haplotypes are different) was 83.6% (± 10.9 SD). The within-locality diversity is given in Table 1. Basically, a single haplotype was observed in each locality except in Kottigehara 1, where three haplotypes were identified (Table 1). The sequencing of an individual in Attigundi 2 and Attigundi 3 confirmed that the SSCP haplotype observed in the two sites was identical.

### *Tests for linkage disequilibrium and Hardy–Weinberg equilibrium*

Of 182 tests of linkage disequilibrium, only 10 were significant ($0.007 < P < 0.05$). Given that 10 tests could be significant by chance alone, we considered the eight microsatellites to be independent. Ten of the 56 probability tests for Hardy–Weinberg equilibrium were significant at $P < 0.05$ (details in Appendix I). This is higher than would be expected by chance alone on the basis of type I errors. Eight of these significant tests were associated with a deficit of heterozygotes but they did not concern specific loci or localities. Only two of these tests were highly significant ($P < 0.0001$) and remained significant after a Bonferroni correction (Sokal & Rohlf 1995). The deficit in heterozygotes in Kottigehara 1 for the loci DC 18 could be explained by a Wahlund effect (see below) and there could be a null allele for the DC 52 locus in Nagu 2. However, any null allele would have a low frequency because amplification was obtained for all individuals. In general, these data suggest a general lack of extensive inbreeding. This agrees with behavioural observations on laboratory colonies in which sister–brother mating was never seen (V. Cuvillier-Hot, pers. com.).

### *Population viscosity (microgeographical genetic differentiation within Kottigehara 1)*

Genetic structure within Kottigehara 1 was first investigated by testing for genic differentiation between the three identified demes (see Fig. 2). For the microsatellite data, although the average value of $F_{ST}$ over all loci was low (0.0195), the exact test of genic differentiation was highly significant ($P = 0.0002$). When comparing each pair of demes, the exact tests of genic differentiation were significant ($P < 0.005$) for the two comparisons involving deme 3. The value of $F_{ST}$ was higher between the two demes which are the furthest apart ($F_{ST}$ between deme 1 and 3 = 0.036) than between demes 1 and 2 ($F_{ST} = 0.015$) and demes 2 and 3 ($F_{ST} = 0.019$).

The exact test of mtDNA genic differentiation between demes was also highly significant ($P < 0.0001$). The geographical structure of the three haplotypes is shown in Fig. 2. The genetic structure observed for mtDNA was approximately 15 times higher than that for the microsatellites, whatever the distance used (equidistant $\Phi_{ST} = 0.33$; euclidean $\Phi_{ST} = 0.41$). When comparing each pair of demes, the exact tests of genic differentiation were significant ($P < 0.004$) for the two comparisons involving deme 2. The lowest value of $\Phi_{ST}$ was between demes 1 and 3 (equidistant and euclidean $\Phi_{ST} = 0.08$), while the higher values were obtained between demes 1 and 2 (equidistant and euclidean $\Phi_{ST} = 0.37$) and between demes 2 and 3 (equidistant $\Phi_{ST} = 0.31$; euclidean $\Phi_{ST} = 0.42$).

The results obtained by estimating the relatedness among neighbouring colonies also showed contrasting results between the two markers. The relatedness among neighbouring colonies was low and nonsignificant different from 0 for the entire locality ($r = 0.07$; *t*-test = 1.58, d.f. = 97, $P > 0.05$) and within deme 2 ($r = 0.035$; *t*-test = 0.72, d.f. = 70, $P > 0.05$) for microsatellites. On the other

hand, using mitochondrial data, the relatedness among neighbouring colonies was high and significantly different from 0 for both the entire locality ($r = 0.74$; $t$-test = 7.03, d.f. = 88, $P < 10^{-5}$) and within deme 2 ($r = 0.68$; $t$-test = 4.7, d.f. = 67, $P < 10^{-5}$). Note that for mtDNA, the expected relatedness between individuals from the same matriline is just one whereas the expected relatedness between individuals from different matrilines is just 0. Therefore the high mtDNA relatedness detected here indicates that neighbouring colonies share in general the same matriline and therefore that mtDNA haplotypes showed a strong spatial clustering as seen in Fig. 2.

In agreement with the results on relatedness, no pattern of isolation by distance was detected within deme 2 (which contains a sufficient number of colonies to perform the analysis) for the microsatellite data. The Spearman rank correlation coefficient between genetic differentiation between individuals and the logarithm of geographical distance was not significant (Mantel test $P > 0.05$). The slope of the regression was negative and close to 0 ($b = -0.0027$).

These results revealed a strong population viscosity for the mtDNA at a fine spatial scale while such population viscosity is low (in the entire site) or nonexistent (in deme 2) for the microsatellite markers.

### *Genetic differentiation between localities*

Exact tests of genic differentiation computed across all localities were highly significant at each microsatellite locus, as well as over all loci ($P < 10^{-5}$). Combined probability tests were also highly significant ($P < 10^{-5}$) for all pairs of localities. In agreement with this high genetic heterogeneity, the values of $F_{ST}$, and $R_{ST}$ over all localities were high and varied among loci from 0.067 (DC18) to 0.42 (DC8), and from 0.03 (DC5) to 0.72 (DC29) for $R_{ST}$. The value of $R_{ST}$ tended to be larger than the value of $F_{ST}$ (Wilcoxon's signed rank test, $Z = 1.96$, $P = 0.049$). When using hypervariable loci $F_{ST}$ is known to underestimate levels of genetic differentiation, with its maximal possible value being constrained by the average expected within-sample homozygosity (Hedrick 1999). In our study, 63% of the variation of $F_{ST}$ among loci was explained by variation in the average expected homozygosity (Spearman's rank correlation $r_s = 0.833$, $N = 8$, $P = 0.01$), whereas such a correlation was not significant when performed with $R_{ST}$ ($r_s = 0.095$, $N = 8$, $P > 0.05$). The significant correlation for $F_{ST}$ indicates that $F_{ST}$ values are influenced strongly by the extent of within-population genetic diversity, as mentioned by Charlesworth (1998). This emphasizes the importance of taking into account the level of genetic diversity when comparing $F_{ST}$ values either between markers or between set of populations.

The results of the hierarchical analysis of molecular variance are given in Table 2 for microsatellite and mitochondrial markers. For both markers, significantly positive estimates of the genetic structure were obtained for both hierarchical levels. The fixation indices for mtDNA were two to three times larger than those obtained for microsatellites across all localities and five to six times larger between localities within region for both types of estimates.

If we consider each region separately, using microsatellites, both $F$- and $R$-statistics varied according to the region considered. They were largest in Attigundi ($F_{ST} = 0.29$; $R_{ST} = 0.31$) followed by Kottigehara ($F_{ST} = 0.10$; $R_{ST} = 0.001$) and Nagu ($F_{ST} = 0.05$; $R_{ST} = 0.006$), with all values statistically significant. Given that three localities were included in Attigundi, we also investigated the genetic differentiation between each pair of localities in Attigundi. A high and significant level of genetic differentiation was detected for both statistics whatever the pair of localities considered (between Attigundi 1 and 2: $F_{ST} = 0.29$; $R_{ST} = 0.26$; between Attigundi 1 and 3: $F_{ST} = 0.25$; $R_{ST} = 0.39$; between Attigundi 2 and 3: $F_{ST} = 0.34$; $R_{ST} = 0.23$).

We did not perform such an analysis using mtDNA, as most localities were characterized by a single unique haplotype. Interestingly, the two localities sharing the same mitochondrial haplotype were in the Attigundi region, which was the region characterized by the greatest amount of microsatellite genetic differentiation.

**Table 2** Hierarchical analysis of molecular variance for microsatellite and mitochondrial markers using the number of different alleles, $F_{ST}$ or haplotypes (equidistant $\Phi_{ST}$) and the sum of squared size difference of alleles, $R_{ST}$, or a distance measure taking into account the haplotype sequences (euclidean $\phi_{ST}$). The significance of the fixation indices was tested using the nonparametric approach of Excoffier *et al.* (1992), based on more than 1000 permutations of individuals (or haplotypes) among localities and among regions or within region according to the hierarchical level tested

| | Microsatellites | | Mitochondrial | |
|---|---|---|---|---|
| | $F_{ST}$ | $R_{ST}$ | Equidistant $\Phi_{ST}$ | Euclidean $\Phi_{ST}$ |
| Across all localities | 0.26 | 0.38 | 0.75 | 0.97 |
| Between localities within region | 0.15 | 0.14 | 0.75 | 0.93 |

All values significant at $P < 0.0001$.

Using all localities, a significant isolation by distance was detected for microsatellites using both *F*-statistics (Mantel test between the logarithm of distance and $F_{ST}/(1 - F_{ST})$; $P = 0.04$) and *R*-statistics (Mantel test between the logarithm of distance and $R_{ST}/(1 - R_{ST})$; $P = 0.04$).

The genetic relationships between mtDNA haplotypes are reflected in the minimum spanning tree and the neighbour-joining tree in Fig. 3. Both trees showed that the haplotypes from Kottigehara and Attigundi regions clustered together, as did the three haplotypes of Kottigehara 1. On the other hand, the two haplotypes from Nagu are highly divergent. Moreover, the Kottigehara haplotypes appeared to be clearly separated from those of the other regions.

### *Male and female gene flow*

The estimates of male and female gene flow are given in Table 3 for the different spatial scales (between demes within Kottigehara 1, between localities within region and across all localities). These values should not be considered

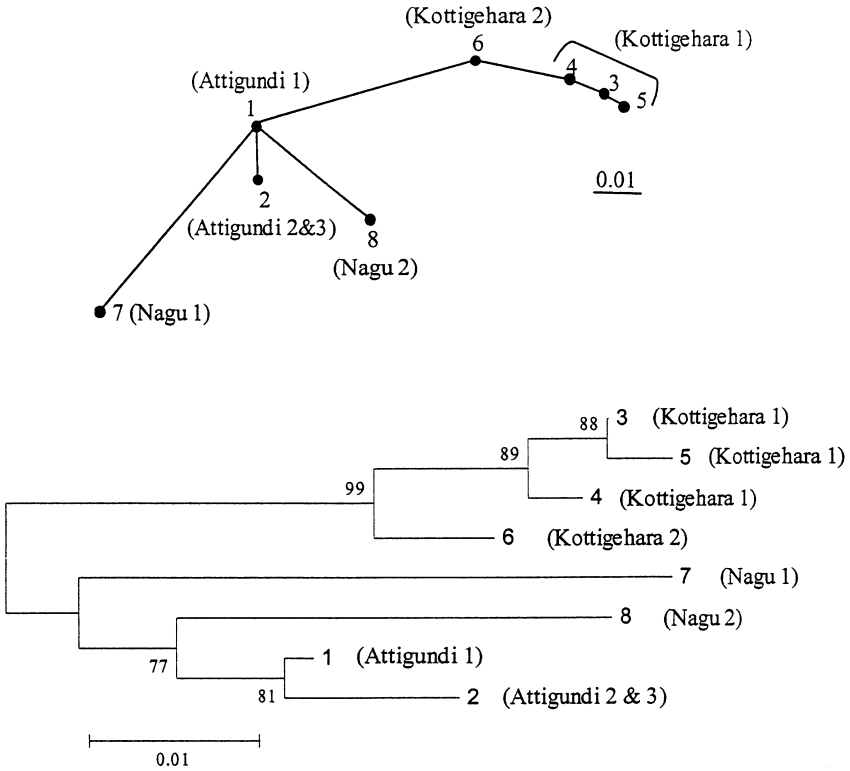


**Fig. 3** Minimum spanning tree (top) and neighbour-joining tree (bottom) reflecting the mtDNA haplotypes relationships. Both trees were based on the genetic distance between mitochondrial sequences using the Jukes and Cantor correction. For the neighbour joining tree, bootstrap values are obtained from 500 iterations.

**Table 3** Estimates of male ($Nm_m$) and female ($Nm_f$) gene flow, derived for the three spatial scales from the estimates of mitochondrial and microsatellite $F_{ST}$. For the two largest spatial scales, we also used $R_{ST}$ estimates from microsatellites. The estimates of $Nm_m$ and $Nm_f$ assume that dispersal occurs before mating and take into account the haplodiploidy (see Materials and methods section for more details), allowing a direct estimation of the relative male and female gene flow

| | $Nm_m$ | | $Nm_f$ | |
|---|---|---|---|---|
| Spatial scale | From $F_{ST}$ | From $R_{ST}$ | From equidistant $\phi_{ST}$ | From euclidean $\phi_{ST}$ |
| Across all localities | 2.2 | 1.6 | 0.3 | 0.03 |
| Between localities within region | 5 | 6 | 0.3 | 0.07 |
| Between demes within Kottigehara 1 | 46.2 | — | 2.0 | 1.44 |

– Rst was not estimated at this lower spatial scale as the mutational process should not matter at this scale.

as 'true' estimates of the dispersal given the strong assumptions underlying the infinite island model (Bossart & Prowell 1998; Hedrick 1999; Whitlock & McCauley 1999). However, whatever the fixation index used, these estimates show clearly that female gene flow is very restricted or even nonexistent, regardless of the spatial scale considered, whereas male gene flow is efficient at small spatial scales but restricted at larger scales.

## Discussion

### *Restricted female gene flow (mtDNA)*

Because of the absence of a winged queen caste in *D. cyaneiventre*, a low level of gene flow is expected via female dispersal. The genetic structure observed for mtDNA strongly supports this hypothesis, as a highly significant genetic differentiation was observed at all spatial scales; within a deme (less than 100 m), between demes within a locality (a few hundred metres), between localities within region (few kilometres) and between all localities (up to 188 km). Basically, the estimates of female gene flow between-localities are lower than 1, regardless of the spatial scale considered. This suggests that female gene flow is not sufficient to prevent the divergence of the mtDNA due to mutational events. In agreement with this, differentiation between-localities at both spatial scales was larger when the degree of difference between the haplotypes was taken into account (euclidean distance). The genetic tree (Fig. 3) showed a phylogeographical structure of the mtDNA haplotypes. The three haplotypes of Kottigehara 1 and the haplotypes of localities from the same region clustered together (except for Nagu), suggesting that occasional migration events occur between nearby localities.

Interestingly, a very low genetic diversity was observed within localities. In fact, seven of eight localities contained only one haplotype, suggesting that new sites are colonized by a single migration event followed by a succession of colony buddings. Such very low genetic variability within localities for mtDNA is expected if colonization events are kin-structured and if migration after colonization is performed mainly by dispersing males (Wade *et al*. 1994). Even though each colonization event implies a strong bottleneck, the trace of this bottleneck will persist longer in cytoplasmic diversity than in nuclear diversity, especially when migration is mainly performed by males (Wade *et al*. 1994). This may explain why the genetic diversity of microsatellite markers in our study was not low. All these results suggest a metapopulation dynamic of mtDNA with extinction of populations and colonization of new sites by adjacent populations.

A very strong genetic differentiation of mtDNA has also been observed in the ant *Formica exsecta* between pastures separated by 1–6 km (Liautard & Keller 2001). Interestingly, *F. exsecta* queens can fly but do not seem to do so in nature. At a similar scale the value of genetic differentiation of mtDNA in the other studies on ants with dependent colony foundation is slightly lower, although highly significant (Ross & Shoemaker 1997; Ross *et al*. 1997; Tay *et al*. 1997; Goodisman & Ross 1998). All these studies show that dependent colony foundation in ants strongly restricts the dispersal ability of females.

### *Population viscosity for nuclear DNA*

Restricted female dispersal due to dependent colony foundation is supposed to induce some population viscosity, i.e. an increase of genetic similarity between neighbouring colonies. Population viscosity using nuclear markers has often been detected in polygynous species in which dependent colony foundation predominates (Pamilo 1983; Crozier *et al*. 1984; Crozier & Pamilo 1986; Seppä & Pamilo 1995; Chapuisat *et al*. 1997; Beye *et al*. 1998). Interestingly, in the queenless ant *Rhytidoponera metallica*, in which colonies contain many mated reproductive workers, no population viscosity was detected (Chapuisat & Crozier 2001). However, the very low genetic relatedness estimated within nests strongly restricted the possibility to detect population viscosity. In this context, our study is informative and novel because *D. cyaneiventre* has a dependent colony foundation but is monogynous with a single nest per colony. We did not detect any population viscosity using nuclear markers at a very fine spatial scale (within a deme of 300 m × 150 m). This indicates an effective dispersal by males which is in agreement with the general lack of inbreeding observed in the populations studied. However, we did observe a significant but low genetic differentiation between demes in Kottigehara 1. This emphasizes the importance of scale and habitat heterogeneity when looking for population viscosity, as noted by Boomsma *et al*. (1990).

### *Genetic differentiation among localities for nuclear DNA*

In *D. cyaneiventre*, restricted female and male dispersal is associated clearly with very high levels of genetic differentiation between localities. Interestingly, the level of genetic differentiation was three to six times larger in Attigundi than in the two other regions. Different explanations can be put forward, most of them not exclusive. First, these differences might be due to an underestimation of genetic differentiation in Kottigehara and Nagu. However, this is unlikely given that the level of differentiation in these regions was probably too low to be constrained by the level of genetic diversity. Second, male gene flow in the Attigundi region might be more limited. This could be due to environmental factors but the habitat

did not appear to be more fragmented than in the other regions. Lower male gene flow could also result from a lower rate of gamergate replacement (see below). A third explanation could be a higher level of genetic drift due to lower effective population size. Indeed, the restricted sample size in the Attigundi localities (see Table 1) reflects the low density of colonies in these areas. Finally, different population histories between regions could also result in variations in the level of genetic differentiation.

Whatever these differences between regions, genetic differentiation is very high in *D. cyaneiventre*. The few studies that have been made of large-scale genetic differentiation in ants have also detected significant genetic differentiation using nuclear markers, but the $F_{ST}$ values were generally lower (Ward 1980; Pamilo 1982; Ross & Shoemaker 1997; Ross *et al*. 1997). For instance, the fixation indices between isolated populations of *F. cinerea* in northern Europe were 0.11 ($F_{ST}$) and 0.08 ($R_{ST}$) (Goropashnaya *et al*. 2001), two to four times lower than in our study. The values estimated in our study are more of the order of those obtained from microsatellite data when comparing populations from different continents (e.g. Franck *et al*. 1998 for honey bees; and Paetkau *et al*. 1999; Goodman *et al*. 2001; for large mammals). With such levels of genetic differentiation, substantial homoplasy might underestimate the genetic differentiation due to the high mutation rates and finite number of unique alleles in microsatellites (Jarne & Lagoda 1996). However, the main result indicating that localities are strongly genetically differentiated cannot be questioned.

Such very restricted gene flow might favour processes of behavioural divergence. A population of *Diacamma* in the south of India, called *D. 'nilgiri'*, was discovered which differs by a social behaviour regulating reproduction (Peeters *et al*. 1992) from its closest relative *D. ceylonense* (C. Peeters, unpublished data, http://www.biologie.ens.fr/fr/ecologie/phylogenie.html). The genetic differentiation between these two taxa is currently being analysed. A large number of *Diacamma* species are found in the southeast Asia and most of them seem to show a restricted geographical distribution (C. Peeters, unpublished data). Even though we lack precise information on these distributions, this might reflect a high rate of speciation in this genus of queenless ants which could be due to their very restricted female dispersal abilities.

### *Male-biased gene flow*

The comparison of genetic structures observed using mtDNA and microsatellites makes it possible to assess the extent of sex-biased dispersal (Ennos 1994; McCauley 1995). Even without sex-biased dispersal, higher population genetic structure is expected with mtDNA markers because of the smaller effective population size and the correspondingly larger susceptibility to genetic drift, compared to nuclear markers (Chesser & Baker 1996). In our study, the discrepancy of genetic structure observed between the two markers is so strong that it probably reflects true differences in dispersal pattern between the two sexes, and in particular a very restricted female gene flow (see above).

Male gene flow is clearly lower between localities than between demes within a locality. Such a decrease of male gene flow with increasing geographical distance was also detected in the marginally significant test of isolation by distance. This result should, however, be interpreted with caution. Localities separated by high levels of genetic differentiation might be more likely to be at a mutation/drift than a migration/drift equilibrium, as the mutation rate can be high compared to the migration rate, especially for microsatellite markers (Goldstein *et al*. 1995). This could lead to an overestimation of gene flow between regions if homoplasy is important (Gaggiotti *et al*. 1999; Balloux *et al*. 2000). In this case, the lower estimate of gene flow based on $R_{ST}$ might be more reliable than that based on $F_{ST}$ (Gaggiotti *et al*. 1999). From these gene flow estimates, we can say that within a locality, male gene flow is about 20–30 times higher than female gene flow (see Table 3). At larger spatial scales, estimating the relative male-biased dispersal does not make sense given that female gene flow is likely to be simply nonexistent (see above).

Direct estimates of male ant dispersal ability are very scarce, and nothing is known about the flying abilities of male *Diacamma*. In laboratory experiments on *S. invicta*, males flew at approximately 2 m/s for a maximum of 30 min (Vogt *et al*. 2000). This suggests that males may rely on passive flight for long-distance dispersal (more than a few kilometres). However, male dispersal does not necessarily imply gene flow. The availability of future gamergates willing to mate is a prerequisite of male gene flow. This situation occurs only after the replacement of the gamergate or after colony fission. The genetic trace of gamergate replacement has been detected in a genetic analysis of colonies collected at Kottigehara 1 by André *et al*. (2001), and the frequency of gamergate replacement was indirectly estimated in this locality to be around 0.8 per colony per year (C. Doums unpublished data). The temporal and spatial variability of the frequency of gamergate replacement is still unknown but might be crucial parameters affecting male gene flow.

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This study is part of a research programme on the behaviour and genetics of queenless *Diacamma* performed in collaboration with the group of R. Gadagkar in Bangalore (India). Claudie Doums is a molecular evolutionary biologist with a particular interest in social insects. At the time of this study, Humberto Cabrera was a masters student undertaking the genetic screening of individuals using microsatellites. Christian Peeters studies the behavioural regulation of reproduction in queenless ants and conducted fieldwork in this study.

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## Appendix I

Observed allele frequency distributions of eight microsatellite loci in seven Indian localities of *Diacamma cyaneiventre*. Alleles are identified by the size (bp) of the amplified fragment. The number of chromosomes successfully screened per locus is given between parenthesis for each locality. Loci are further described in Doums (1999). The gene diversity was calculated using an unbiased estimator (Nei 1987; 164)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Locus DC5 | Allele (bp) | Attigundi 1 (30) | Attigundi 2 (24) | Attigundi 3 (24) | Kottigehara 1 (200) | Kottigehara 2 (40) | Nagu 1 (48) | Nagu 2 (54) |
| | 137 | | | | | | | 0.037 |
| | 141 | 0.200 | | | | | | |
| | 143 | | 0.042 | | 0.165 | 0.025 | 0.021 | |
| | 145 | 0.021 | | | | | | |
| | 147 | 0.500 | 0.250 | 0.708 | 0.255 | 0.125 | 0.625 | 0.407 |
| | 149 | 0.033 | 0.708 | 0.250 | 0.580 | 0.850 | 0.250 | 0.537 |
| | 151 | 0.267 | | 0.042 | | | 0.083 | 0.019 |
| | Gene diversity | 0.657 | 0.447 | 0.451 | 0.574 | 0.267 | 0.554 | 0.552 |
| | $F_{IS}$ | −0.116* | −0.305 | −0.109 | 0.007 | −0.123 | 0.324† | −0.208 |
| Locus DC8 | Allele (bp) | Attigundi 1 (30) | Attigundi 2 (24) | Attigundi 3 (24) | Kottigehara 1 (192) | Kottigehara 2 (40) | Nagu 1 (46) | Nagu 2 (54) |
| | 128 | 0.167 | | | | | | |
| | 130 | | | | | | 0.022 | |
| | 132 | | | | | | 0.304 | 0.074 |
| | 134 | | 0.167 | 0.042 | | | 0.022 | |
| | 136 | 0.633 | | | 0.005 | | | 0.056 |
| | 138 | 0.033 | 0.042 | | 0.089 | 0.100 | 0.500 | 0.722 |
| | 140 | | | | 0.089 | | 0.130 | 0.111 |
| | 142 | 0.167 | 0.250 | 0.917 | 0.807 | 0.600 | 0.022 | 0.019 |
| | 144 | | | | 0.010 | 0.300 | | 0.019 |
| | 146 | | 0.458 | 0.042 | | | | |
| | 148 | | 0.083 | | | | | |
| | Gene diversity | 0.557 | 0.716 | 0.163 | 0.334 | 0.550 | 0.658 | 0.465 |
| | $F_{IS}$ | −0.197 | −0.164 | −0.023 | 0.003 | −0.273 | 0.339 | −0.035 |
| Locus DC11 | Allele (bp) | Attigundi 1 (28) | Attigundi 2 (24) | Attigundi 3 (24) | Kottigehara 1 (202) | Kottigehara 2 (40) | Nagu 1 (42) | Nagu 2 (54) |
| | 205 | | | | 0.005 | | | |
| | 209 | 0.036 | 0.042 | | | | | 0.019 |
| | 211 | | | | | | | 0.019 |
| | 213 | | | | 0.005 | 0.150 | | |
| | 215 | | | | 0.030 | 0.100 | | |
| | 219 | 0.643 | 0.208 | 0.273 | 0.574 | 0.100 | 0.024 | |
| | 221 | 0.143 | 0.375 | 0.136 | 0.069 | 0.225 | 0.286 | 0.093 |
| | 223 | | | | | | 0.024 | 0.019 |
| | 225 | | | | | | 0.024 | 0.037 |
| | 227 | | | | 0.010 | 0.200 | 0.024 | 0.056 |
| | 229 | 0.143 | | | 0.020 | 0.025 | 0.071 | |
| | 231 | | | 0.455 | 0.035 | 0.025 | 0.048 | 0.019 |
| | 233 | | 0.25 | | 0.025 | | 0.048 | 0.111 |
| | 235 | | | | | | 0.095 | 0.019 |
| | 237 | | | | 0.040 | 0.025 | 0.048 | 0.093 |
| | 239 | | | | 0.005 | 0.025 | 0.048 | 0.074 |
| | 241 | | | | 0.005 | | 0.048 | 0.130 |
| | 243 | 0.036 | | | 0.025 | | 0.024 | 0.019 |
| | 245 | | | | 0.005 | | 0.024 | |
| | 247 | | | | | | 0.071 | 0.074 |
| | 249 | | | | 0.005 | 0.050 | 0.024 | 0.074 |
| | 251 | | | 0.045 | 0.129 | | | 0.019 |
| | 253 | | 0.083 | | 0.015 | 0.075 | 0.048 | 0.037 |
| | 255 | | | 0.045 | | | | |
| | 257 | | | 0.045 | | | | 0.037 |
| | 259 | | 0.042 | | | | | 0.056 |
| | 263 | | | | | | 0.024 | |
| | Gene diversity | 0.566 | 0.792 | 0.723 | 0.646 | 0.879 | 0.904 | 0.945 |
| | $F_{IS}$ | 0.117 | 0.474† | −0.132 | 0.004 | 0.033 | 0.051 | 0.177* |

**Appendix I** *Continued*

| Locus DC18 | Allele (bp) | Attigundi 1 (30) | Attigundi 2 (22) | Attigundi 3 (24) | Kottigehara 1 (146) | Kottigehara 2 (40) | Nagu 1 (22) | Nagu 2 (52) |
|---|---|---|---|---|---|---|---|---|
| | 182 | | | | | | | 0.019 |
| | 184 | 0.067 | | | | | | |
| | 188 | 0.033 | | | | | | |
| | 190 | 0.033 | | | | | | 0.019 |
| | 194 | | | | 0.007 | | | |
| | 198 | 0.033 | | | 0.041 | 0.075 | | |
| | 200 | 0.033 | | | 0.075 | | | |
| | 202 | 0.133 | | | 0.096 | | 0.045 | |
| | 204 | | | | 0.034 | | | |
| | 206 | | | | 0.021 | | | |
| | 208 | | | | 0.014 | 0.025 | 0.045 | |
| | 210 | | | | 0.021 | 0.050 | | |
| | 212 | | | | 0.240 | 0.050 | 0.136 | |
| | 214 | 0.033 | 0.045 | | | 0.125 | | 0.045 |
| | 216 | 0.133 | 0.136 | | 0.014 | 0.125 | 0.096 | |
| | 218 | 0.033 | 0.045 | 0.167 | 0.021 | | | 0.019 |
| | 220 | | 0.091 | | 0.048 | 0.050 | | 0.077 |
| | 222 | | 0.045 | 0.042 | | 0.400 | 0.045 | 0.115 |
| | 224 | 0.133 | 0.091 | 0.167 | | | | 0.115 |
| | 226 | | 0.091 | 0.083 | 0.007 | | 0.182 | 0.058 |
| | 228 | 0.167 | 0.091 | | 0.048 | | | 0.154 |
| | 230 | | 0.318 | 0.042 | 0.007 | | | 0.058 |
| | 232 | | | | | 0.050 | 0.045 | 0.019 |
| | 234 | | 0.045 | | 0.062 | | | 0.019 |
| | 236 | 0.067 | | | 0.048 | 0.050 | 0.227 | 0.038 |
| | 238 | 0.067 | | 0.083 | 0.096 | | 0.091 | 0.019 |
| | 240 | | | | 0.041 | | | |
| | 242 | | | | 0.021 | | | 0.038 |
| | 244 | | | 0.083 | | | 0.045 | 0.019 |
| | 246 | | | | 0.007 | | | |
| | 248 | | | | 0.014 | | | |
| | 250 | | | 0.042 | | | | 0.077 |
| | 252 | | | 0.042 | 0.014 | | | 0.019 |
| | 254 | | | | 0.007 | | 0.045 | |
| | 256 | | | 0.042 | | | 0.045 | |
| | 260 | | | 0.042 | | | | 0.019 |
| | 262 | 0.033 | | | | | | |
| | 266 | | | 0.167 | | | | |
| | Gene diversity | 0.926 | 0.873 | 0.920 | 0.908 | 0.814 | 0.927 | 0.935 |
| | $F_{IS}$ | −0.080 | −0.146 | −0.086 | 0.125‡ | 0.202* | 0.314 | 0.053 |
| Locus DC19 | Allele (bp) | Attigundi 1 (30) | Attigundi 2 (20) | Attigundi 3 (22) | Kottigehara 1 (194) | Kottigehara 2 (36) | Nagu 1 (42) | Nagu 2 (56) |
| | 221 | | | | | | 0.333 | 0.607 |
| | 223 | | | | | | 0.357 | 0.054 |
| | 225 | | | | 0.005 | | | |
| | 229 | | 0.050 | | 0.082 | 0.083 | 0.167 | 0.071 |
| | 231 | 0.033 | | | | | 0.024 | 0.036 |
| | 233 | 0.200 | | | 0.242 | | 0.024 | 0.107 |
| | 235 | | | 0.182 | 0.165 | 0.250 | | |
| | 237 | 0.633 | 0.100 | 0.091 | 0.196 | 0.139 | 0.095 | 0.107 |
| | 239 | 0.067 | | 0.136 | 0.021 | 0.056 | | |
| | 241 | | | 0.273 | 0.253 | 0.222 | | |
| | 243 | | 0.300 | | 0.036 | 0.028 | | |
| | 245 | 0.067 | | 0.273 | | 0.222 | | 0.018 |
| | 247 | | | 0.045 | | | | |
| | 249 | | 0.150 | | | | | |
| | 253 | | 0.150 | | | | | |
| | 255 | | 0.150 | | | | | |
| | 257 | | 0.100 | | | | | |
| | Gene diversity | 0.574 | 0.867 | 0.827 | 0.808 | 0.833 | 0.739 | 0.608 |
| | $F_{IS}$ | 0.303 | 0.077 | 0.011 | 0.030† | 0.067 | −0.095 | −0.175 |

**Appendix I** *Continued*

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Locus DC20 | Allele | Attigundi | Attigundi | Attigundi | Kottigehara | Kottigehara | Nagu | Nagu |
| | (bp) | 1 (30) | 2 (24) | 3 (24) | 1 (200) | 2 (36) | 1 (48) | 2 (56) |
| | 152 | | | | 0.005 | | 0.021 | 0.054 |
| | 154 | | | | 0.005 | | | |
| | 156 | | | | 0.115 | 0.167 | 0.062 | 0.018 |
| | 158 | | | | | 0.139 | 0.854 | 0.679 |
| | 160 | 0.400 | 0.958 | 0.083 | 0.235 | 0.472 | 0.062 | 0.214 |
| | 162 | 0.500 | 0.042 | 0.917 | 0.360 | 0.167 | 0.018 | |
| | 164 | 0.100 | | | 0.155 | 0.056 | | 0.018 |
| | 166 | 0.125 | | | | | | |
| | Gene diversity | 0.595 | 0.083 | 0.159 | 0.766 | 0.717 | 0.270 | 0.501 |
| | $F_{IS}$ | −0.232 | 0.000 | −0.048 | −0.018 | −0.084 | 0.383* | 0.215 |
| Locus DC29 | Allele | Attigundi | Attigundi | Attigundi | Kottigehara | Kottigehara | Nagu | Nagu |
| | (bp) | 1 (28) | 2 (22) | 3 (24) | 1 (200) | 2 (38) | 1 (46) | 2 (54) |
| | 208 | | | | 0.110 | 0.211 | | |
| | 212 | 0.036 | | 0.417 | 0.075 | 0.132 | | |
| | 214 | 0.821 | 0.318 | 0.500 | 0.815 | 0.658 | 0.087 | 0.148 |
| | 216 | 0.143 | 0.682 | 0.083 | | | 0.109 | 0.019 |
| | 218 | | | | | | 0.022 | 0.019 |
| | 220 | | | | | | 0.304 | 0.185 |
| | 222 | | | | | | 0.283 | 0.444 |
| | 224 | | | | | | 0.065 | 0.167 |
| | 226 | | | | | | 0.043 | |
| | 228 | | | | | | 0.022 | 0.019 |
| | 230 | | | | | | 0.065 | |
| | Gene diversity | 0.313 | 0.464 | 0.595 | 0.320 | 0.519 | 0.815 | 0.729 |
| | $F_{IS}$ | −0.140 | 0.412 | 0.019 | 0.093 | −0.014 | 0.040 | −0.169 |
| Locus DC52 | Allele | Attigundi | Attigundi | Attigundi | Kottigehara | Kottigehara | Nagu | Nagu |
| | (bp) | 1 (30) | 2 (24) | 3 (24) | 1 (198) | 2 (40) | 1 (48) | 2 (56) |
| | 147 | | | | | | | 0.125 |
| | 149 | | | | | | | 0.411 |
| | 155 | | | | | | 0.521 | 0.161 |
| | 157 | | | | | | 0.042 | 0.304 |
| | 161 | | 0.750 | | | 0.175 | 0.417 | |
| | 163 | 0.433 | | 0.083 | 0.202 | | | |
| | 165 | | | | | 0.250 | 0.021 | |
| | 167 | | 0.167 | 0.708 | | 0.200 | | |
| | 169 | 0.200 | 0.042 | 0.208 | 0.414 | 0.025 | | |
| | 171 | 0.267 | 0.042 | | 0.045 | | | |
| | 173 | 0.033 | | | 0.071 | 0.025 | | |
| | 175 | 0.033 | | | | | | |
| | 177 | 0.033 | | | 0.247 | 0.125 | | |
| | 179 | | | | | 0.050 | | |
| | 181 | | | | | 0.150 | | |
| | 189 | | | | | | | |
| | Gene diversity | 0.717 | 0.428 | 0.477 | 0.723 | 0.846 | 0.563 | 0.714 |
| | $F_{IS}$ | −0.209 | 0.221 | 0.476 | 0.036 | −0.005 | −0.109* | 0.300‡ |

*$P < 0.05$; †$P < 0.005$; ‡$P < 0.0009$ the threshold value after a Bonferonni correction.