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Genetic variability in the social bee *Lasioglossum marginatum* and a cryptic undescribed sibling species, as detected by DNA fingerprinting and allozyme electrophoresis

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Abstract

DNA fingerprints (DNAfp) were obtained for three widely separated samples of bee related to *Lasioglossum marginatum* using the M13 sequence as a probe. Bee samples were obtained from France (three localities separated by at most 20 km), Greece and India. All European populations exhibited almost identical profiles with similarity indices (*S*) of over 98% within a French sample, 94% among Greek bees and 90% between Greek and French bees. The DNAfp profiles of Indian bees showed more polymorphism (intrapopulation *S* = 77%) and were quite dissimilar to the European samples (*S* = 55% and 56% to French and Greek samples, respectively). The similarity between populations separated by over 2000 km is higher than among unrelated individuals within a population in two other bee species and the tsetse fly. Data from allozyme electrophoresis shows parallel variation to that obtained with DNAfp and the genetic differences between Indian and European samples are strikingly large (Indian and European populations shared no alleles at 14 out of 47 loci surveyed) such that no more than one species must be involved. Nonetheless, the samples are indistinguishable morphologically. We argue that chronically low effective population size in these species results in low levels of genetic variability and that this, combined with a genetic bottleneck during the speciation event and colonization of Europe, may have resulted in both the extremely low levels of DNAfp variation in European bees and the large number of fixed allelic differences between European and Indian samples. We conclude that DNAfp may be particularly useful as a tool to study population level phenomena in organisms with only moderate levels of DNAfp variability and that the Hymenoptera may be particularly appropriate model systems for such research.

Keywords: allozyme variations, DNA fingerprinting, *Lasioglossum marginatum*, social bees.

Introduction

DNA fingerprinting (DNAfp) is now commonly used to study genetic variability and to analyse pedigree relationships in a wide variety of organisms including insects (Jeffreys *et al.*, 1985a, b; Jeffreys & Morton, 1987; Vassart *et al.*, 1987; Burke *et al.*, 1989, 1991; Ryskov *et al.*, 1989; Meng *et al.*, 1990; Blanchetot, 1991a, b). The methodology uses genomic DNA hybridized with repeated sequences as probes to detect polymorphisms which result from variation in the number of copies of tandemly repeated DNA sequences. In most species studied to date, the hybridizing banding pattern is highly polymorphic and unique to an individual, hence the term DNA fingerprint (Jeffreys *et al.*, 1985b). In insects, when unrelated individuals are compared, the patterns obtained usually contain some bands which are common to all individuals indicating monomorphism at some loci (Blanchetot, 1991a, b; A. Blanchetot and R. H. Gooding, unpublished data). Monomorphic bands are not useful for estimating genealogical relationships within families. However, comparatively monomorphic profiles among individuals have been shown to be useful for examining relationships among populations (Gilbert *et al.*, 1990; Longmire *et al.*, 1991).

It is generally accepted that Hymenoptera have lower levels of allozyme variation than most other insects, probably as a result of haplodiploidy (see recent reviews by Crespi, 1991; Packer & Owen, 1992). Comparisons of band-sharing estimates among unrelated individuals of two species of Diptera (Blanchetot, 1991a; A. Blanchetot and R. H. Gooding, unpublished data) and two bee species (Blanchetot, 1991b, c) indicate that a similar pattern might

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apply to DNAfp-detected variation. This suggests that the Hymenoptera may be particularly appropriate model organisms for further test of the utility of DNAfp at the population level.

To date, there have been no overt comparisons of intraspecific genetic variation as detected by DNAfp and allozyme analysis. In this paper, we compare the levels of intra- and interpopulation genetic variation detected by these two methods for three widely separated samples of perennially social sweat bee. Initially, all three samples were thought to represent one species—*Lasioglossum marginatum*. However, upon electrophoretic analysis, extremely large genetic distances were found between the one Indian and two European samples such that they cannot represent the same species. All the samples remain morphologically indistinguishable in the characteristics that normally separate closely related halictine species: head shape and cuticular sculpturing in both sexes and the genitalia of males. In the absence of morphological criteria we do not describe a new species herein but refer to all three samples as representing the *Lasioglossum marginatum* species complex with the name *L. marginatum* being restricted to the European samples

Materials and methods

Background Information

The *L. marginatum* species complex is morphologically very distinct, based upon numerous physical characteristics of both sexes. It has a unique social organization which is sufficiently bizarre to require detailed description. The following account is summarized from Quénu (1959). Nests are initiated in spring by solitary females. One worker brood is produced each year for 4 or 5 years and older colonies may contain several hundred workers and the original queen. In the fifth or sixth year, a mixture of haploid and diploid eggs is produced with the workers responsible for much of the haploid egg production. A male biased (approximately 3:1) sex ratio has been reported for this final year's brood. Females remain in their natal brood cell throughout the winter. Upon eclosion the males, unlike their sisters, do not remain in their natal brood cells but reach the soil surface aided by the activities of the surviving workers which dig through the plugged nest burrow. The males search for other open nests, enter them and mate inside the females' natal brood cell; returning to their own natal nest at the end of the day. Thus, only those nests that are open to permit the egress of males are also open to allow the females within to mate; mating determines caste and there are no morphological differences between queens and workers. Nests that produce males die out the following year because all the young females become nest foundresses that start a new nest elsewhere.

A similar social cycle has been reported for populations as far east as Yugoslavia (Grozdanic, 1966). Although nests of the Indian species have not been excavated in India, its phenology and the reproductive physiology of females is consistent with a perennial social cycle and inconsistent with any other type of sociality known in insects (I. Packer, in preparation).

Sample collection

Samples were collected from three different countries: France, Greece and India, as follows. Single females were aspirated from a large number of nests at Villajou in the Dordogne region of France in April 1988. Two other localities in this area were sampled in April 1991: females were collected from flowers at La Mouthe and one female was aspirated per nest from an aggregation at La Combe de la Faye. La Mouthe and Villajou are approximately 20 km distant from one another with La Combe de la Faye equidistant at 10 km from both. Bees were collected from flowers at Monemvasia in southern Greece in spring 1989 and Naggar, Himachal Pradesh, India, in April 1990. Monemvasia is approximately 2100 km from the Dordogne and 5000 km from the Indian sample sites. In each case, bees were collected in an area less than 1 acre, although individuals caught on flowers could have come from nests some distance away. Bees were kept alive until they could be cryogenically preserved.

Fingerprinting protocol

DNA from individual bees was extracted according to the procedure of Blanchetot (1991a). After digestion with *Hae*III, the DNA samples were electrophoresed on a 0.7% agarose gel and transferred onto a nylon membrane. M13 phage DNA was used as a probe and was labelled by hexamer priming using ^{32}P-dCTP to a specific activity of 10^8–10^9 cpm μg^{-1} (Feinberg & Vogelstein 1984). The filters were hybridized with 20 ng ml^{-1} of labelled probe as described by Westneat *et al.* (1988). Autoradiography was carried out at −70°C using Kodak XAR film with intensifying screens. The formulae used to analyse genetic similarity data were taken from Lynch (1990, 1991) with the exception of estimates of variance. Our sample sizes are too small for applications of the inter-individual comparisons recommended by Lynch to give sensible values. Consequently, we present simple standard deviations as variance estimators. Although one should be very cautious of these estimates, the results themselves are quite clear. We ran three gels to compare levels of DNAfp variation. Gel 1 contained nine females from Villajou in France. Gel 2 compared the three French populations with seven bees from La Mouthe, seven from Villajou and four from La Combe de la Faye. The third gel compared bees from the

three countries with seven individuals from Naggar, India, six from Monemvasia, Greece and four from La Combe de la Faye. A replicate sample of eight bees from Villajou was run on a fourth gel.

Electrophoretic methods

Electrophoretic methods were as reported by Packer & Owen (1989, 1990) with minor modifications. One additional locus, acylase, was surveyed in the present study using the stain recipe of Qavi & Kit (1980) and the RSL buffer system (Ridgway *et al.*, 1970). Previous analyses (Packer, 1991; Packer & Owen, 1992; L. Packer, in preparation) indicated almost complete monomorphism at 52 loci for 30 diploid individuals (one per nest in French *L. marginatum* from Villajou). Consequently, only a few additional females from this population were compared with Greek and Indian bees on line-up gels in the present study. Genetic distance and heterozygosity analyses were performed using the BIOSYS program (Swofford & Selander, 1989).

Results

DNAfp

Bees were compared from (i) different nests within single aggregations (Fig. 1), (ii) different aggregations within one region of France (Fig. 2) and (iii) from different countries (Fig. 3).

(i) All nine bees from different nests in Villajou shared the same 15 bands with an additional band present in six individuals to give an average of 15.7 bands per individual. The similarity index (S) among the nine individuals was 98.4 ± 0.3 (n = 36 pairwise comparisons). The replicate sample from Villajou showed 14 or 15 bands with the variable band in a different position than the one marked in Fig. 1 and being found only in one individual. This sample gave almost identical results to the first gel with S = 99.0 ± 0.01 (n = 28 comparisons).

(ii) DNAfp profiles among the three distinct nesting aggregations from France (Fig. 2) are identical. Only 13 bands are readily discernible and all are monomorphic. The variable band detected in Fig. 1 is too poorly resolved on the second gel (Fig. 2) to permit scoring. Nonetheless, it is likely that the genetic differentiation between the three nearby locations is no greater than that found within a population.

(iii) Figure 3 shows that the same monomorphic DNA profile found in French localities extends to Greek bees, suggesting that populations all across southern Europe probably share almost identical DNA fingerprints. The similarity index (S) among Greek bees is 93.9% ± 2.8 (21 pairwise comparisons for an average of 19.4 ± 0.5 bands)

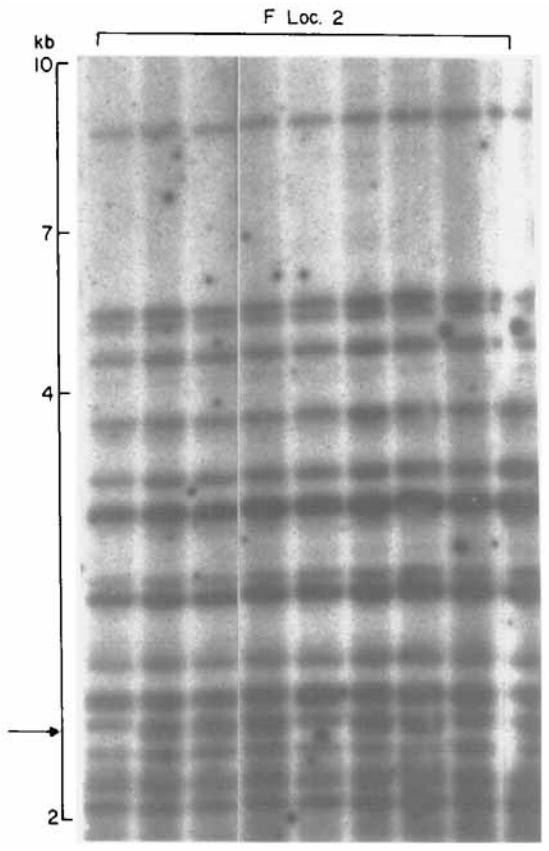

Figure 1. DNAfp profiles of nine *L. marginatum* females each aspirated from a separate nest in an aggregation at Villajou in the Dordogne region of France. The single variable band is indicated with an arrow.

and S between Greek bees and the French ones scored from the same gel is 90.1% (n = 4 comparisons). However, the banding patterns of the Indian sample are quite distinct from the European ones (S = 55.1%, n = 7, and 56.4%, n = 4, to French and Greek bees respectively) and, furthermore, there is a reasonable amount of variation within the Indian sample (S = 76.6% ± 7.2, n = 21 pairwise comparisons with an average of 17.7 ± 1.4 bands per individual).

An alternative way of analysing variation levels among populations is to compare the proportion of bands that are variable. In the French population illustrated in Fig. 1, 15 of 16 bands are monomorphic. For the Greek bees in Fig. 2, 16 out of 21 bands are monomorphic. This difference is not significant (Fishers exact test P = 0.16). In the Indian sample, 17 out of 27 bands are variable giving a χ^2 value of 7.29 ($P < 0.01$) when compared to the most variable European population, that from Greece. Although the statistical measures employed here are not very precise and the sample sizes are small, the results are clear. There is practically no genetic variation at DNAfp loci in the French populations, very little variation among Greek bees but

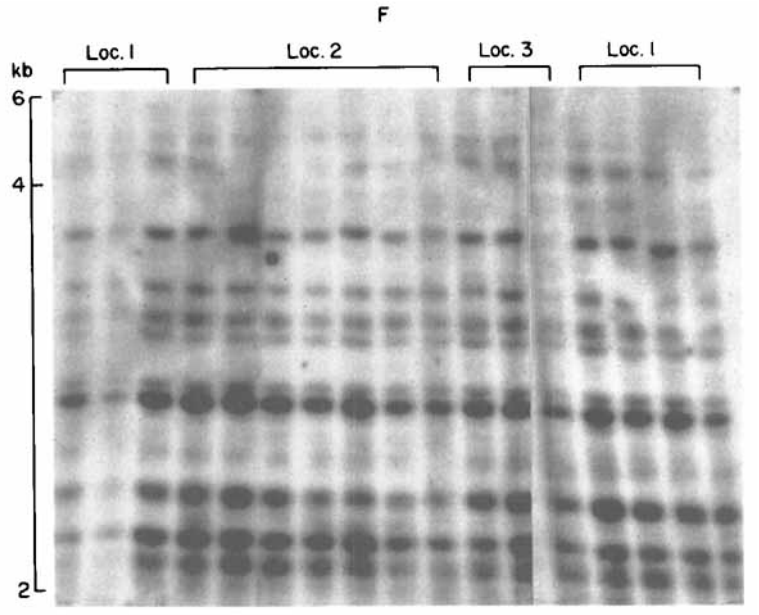

Figure 2. DNAfp profiles for *L. marginatum* from three localities in the Dordogne region of France. Lanes 1–3 and 14–17 are of bees from La Mouthe (loc. 1) collected from flowers, lanes 4–10 are bees from Villajou (loc. 2), one per nest and lanes 11–13 are from La Combe de la Faye (loc. 3), again one bee per nest.

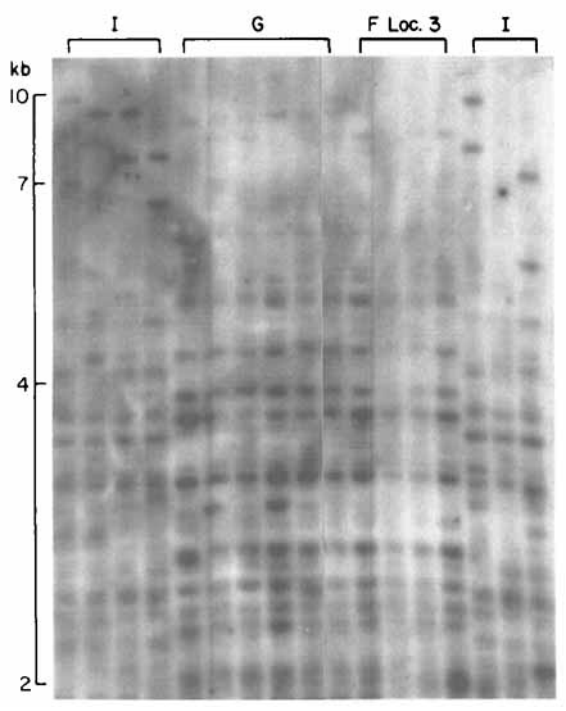

Figure 3. DNAfp profiles of *L. marginatum* from three different countries. Lanes 1–4 and 15–17 are of bees collected from flowers at Naggar, India (I); lanes 5–10 are bees collected from flowers at Monemvasia, Greece (G) and lanes 11–14 were obtained from four bees collected from different nests at La Combe de la Faye, France (F).

reasonable amounts of polymorphism in the Indian sample.

The monomorphism found here, particularly in the European samples, is in stark contrast to that found in other insects by DNAfp. Thus, in unrelated outbred tsetse flies the estimate of S was 35%, 44% in the alfalfa leafcutting bee and 50% in the honeybee (Blanchetot, 1991c; A. Blanchetot and R. H. Gooding, unpublished data). The similarity between samples separated by over 7000 km is higher than among unrelated individuals within single populations of two other bee species and a tsetse fly studied using identical methods.

Electrophoretic data

Table 1 lists the 47 loci surveyed along with their enzyme commission numbers, symbols and running conditions. Table 2 summarizes electromorph mobilities, allele frequencies and single locus heterozygosity estimates.

Table 3 summarizes genetic variation in the three samples. There is a progressive increase in all indicators in genetic variation from the French through the Greek to the Indian population. The expected heterozygosity for the French population is one of the lowest estimates obtained for Hymenoptera and the lowest recorded out of 19 species of halictine surveyed (Packer & Owen, 1989, 1990, 1992; Rosenmeier and L. Packer, unpublished data). Heterozygosity within the Greek population is below average for Hymenoptera with the Indian sample being slightly above average.

The genetic identity between French and Greek bees was very high (0.99) with differences being restricted to allele frequency variation between the two populations. Conversely, the genetic identity between the Indian and both European samples was surprisingly low (0.62 and 0.65 to French and Greek bees respectively) with no fewer than 14 loci sharing no alleles (Table 2). Such a low genetic

Table 1. List of enzyme loci used, number of loci and running conditions.

Enzymes	E.C. number	Symbol	Number of loci	Buffer system*
β-*N*-acetylhexosaminidase	3.2.15.2	*Aha*	1	bI
Acylase	3.5.1.14	*Acy*	1	RSL
Aconitate hydratase	4.2.1.3	*Ac*	2	CAM
Adenylate kinase	2.7.4.3	*Ak*	3	CAM
Alanine aminotransferase	2.6.1.2	*Aat*	1	bV
Aldehyde dehydrogenase	1.2.1.3	*Alddh*	1	bV
Alkaline phosphatase	3.1.3.1	*Akp*	1	bV
Arginine kinase	2.7.3.3	*Ark*	1	bI
Diaphorase (NADPH)	1.6.99*	*Diap*	1	bV
Enolase	4.2.1.11	*Enol*	1	bI
Esterase	–	*Est*	4	RSL
Fructose biphosphate aldolase	4.1.2.13	*FBA*	1	bIII
Glucose-6-phosphate dehydrogenase	1.1.1.49	*G6pd*	2	CAM
Glucose-6-phosphate isomerase	5.3.1.9	*Gpi*	1	bI
α-Glucosidase	3.2.1.20	*Glu*	2	bI
Glyceraldehyde-3-phosphate dehydrogenase	1.2.1.12	*Gaph*	1	bI
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3pd*	2	bI
Guanine deaminase	3.5.4.3	*Gda*	1	bV
Hexokinase	2.7.1.1	*Hk*	1	bI
D-2-Hydroxy-acid dehydrogenase	1.1.99.6	*Had*	1	bI
Hydroxyacylglutathione hydrolase	3.1.2.6	*Hgh*	1	bV
L-Iditol dehydrogenase	1.1.1.14	*Idh*	1	bV
Isocitrate dehydrogenase	1.1.1.42	*Idh*	1	CAM
Malate dehydrogenase	1.1.1.37	*Mdh*	2	CAM
Malate dehydrogenase (NADP)	1.1.1.40	*Me*	1	bI
Peptidase (gly–leu)	3.4.11–13	*Pep(gl)*	1	RSL
Peptidase (leu–ala)	3.4.11–13	*Pep(la)*	2	RSL
Peptidase (phe–pro)	3.4.13.8	*Pep(pp)*	1	RSL
Phosphoglucomutase	5.4.2.2	*Pgm*	1	bI
6-Phosphogluconate dehydrogenase	1.1.1.43	*6Pgd*	1	CAM
Phosphoglycerate kinase	2.7.2.3	*Pgk*	1	bI
Phosphoglyceromutase	5.4.2.1	*Pgam*	1	bI
Pyruvate kinase	2.7.1.40	*Pk*	1	bV
Superoxide dismutase	1.15.1.1	*Sod*	1	CAM
Triose-phosphate isomerase	5.3.1.1	*Tpi*	1	bI
Xanthine dehydrogenase	1.1.1.204	*Xdh*	1	bIII

*bI, bIII and bV from Shaw & Prasad (1970), CAM from Clayton & Tretiak (1972), RSL from Ridgway *et al.* (1970).

identity is not consistent with Indian and European specimens being conspecific, indeed these values are close to the average for non-sibling species of insect (Brussard *et al.*, 1985).

The Greek population is polymorphic at two loci for which alternative alleles are fixed in the French and Indian bees. Thus, the fast allele for *Gpi* is rare in the Greek population, fixed in Indian bees and absent in those from France. Similarly, at *Pgm* the Greek bees are polymorphic but this time the common allele is the one fixed in the Indian sample and lacking in the French population. Thus, the Greek population has genotype arrays consistent with its intermediate geographical position between Indian and French samples.

Discussion

The *L. marginatum* species complex is morphologically very distinct. The head shape of the females, surface sculpturing and pubescence characteristics of the thorax and abdomen, close similarity in overall appearance between males and females (most halictines are extremely sexually dimorphic, separation of the sexes in the species complex requires microscopic examination) and the male genitalia all provide well-defined derived characteristics. These bees also have a unique social organization—they are the only halictines with perennial societies and the only social insect to produce one discrete worker brood per year. Our results indicate that the Indian and European samples are genetically distinct at enzyme-encoding loci. Despite being morphologically and behaviourally indistinguishable. Because of the lack of morphological differences between populations and the unavailability of suitable material for genetic analysis from locations between India and Greece, we consider a formal description of the Indian species to be premature.

The heterozygosity estimates obtained for European samples are low even for Hymenoptera whereas the genetic variability within Indian bees is slightly above average for Hymenoptera (Crespi, 1991; Packer & Owen, 1992).

Table 2. Allele frequencies, individual locus heterozygosity* and sample size data for variable loci.

Locus	Mobility (mm)	Frequency		
		France	Greece	India
Acy	25	0.000	0.000	0.091
	28	1.000	1.000	0.000
	32	0.000	0.000	0.909
	H	0.000	0.000	0.169
	n	13	31	22
Ak-3	15	0.000	0.000	0.088
	20	1.000	0.988	0.912
	25	0.000	0.012	0.000
	H	0.024	0.000	0.000
	n	36	41	17
Aat	10	0.000	0.000	1.000
	16	1.000	1.000	0.000
Alddh	14	1.000	1.000	0.000
	16	0.000	0.000	1.000
Ark	35	1.000	1.000	0.000
	42	0.000	0.000	1.000
Est-1	25	1.000	1.000	0.000
	35	0.000	0.000	0.118
	41	0.000	0.000	0.824
	47	0.000	0.000	0.059
	H	0.000	0.000	0.314
	n	33	36	17
Est-2	27	1.000	1.000	0.000
	36	0.000	0.000	1.000
G6pd-2	15	1.000	1.000	0.000
	17	0.000	0.000	1.000
Gpd-2	35	1.000	1.000	0.977
	39	0.000	0.000	0.023
	H	0.000	0.000	0.045
	n	33	46	22
Gpi	2	1.000	0.989	0.000
	7	0.000	0.011	1.000
	H	0.000	0.022	0.000
	n	43	45	17
Gda	40	0.767	0.700	0.900
	48	0.233	0.300	0.000
	54	0.000	0.000	0.100
	H	0.361	0.434	0.200
	n	43	15	5
Had	3	1.000	0.891	0.000
	8	0.000	0.109	0.000
	15	0.000	0.000	1.000
	H	0.000	0.196	0.000
	n	43	46	22
Hgh	56	1.000	1.000	0.000
	60	0.000	0.000	1.000
Hk-2	18	1.000	1.000	0.000
	22	0.000	0.000	1.000
Idh	12	0.000	0.000	1.000
	18	1.000	1.000	0.000
Me	20	0.000	0.000	0.441
	25	1.000	1.000	0.559
	H	0.000	0.000	0.508
	n	33	31	17
Pep(gl)	19	0.000	0.000	0.023
	26	1.000	1.000	0.977
Pep(gl) cont.	*H*	0.000	0.000	0.045
	n	13	46	22
Pep(la-2)	28	0.000	0.000	1.000
	30	1.000	1.000	0.000
Pep(pp)	50	0.000	0.000	0.412
	52	1.000	1.000	0.588
	H	0.000	0.000	0.499
	n	33	36	17
6Pgd	−2	1.000	1.000	0.000
	−7	0.000	0.000	1.000
Pgm	20	1.000	0.197	0.000
	28	0.000	0.803	1.000
	H	0.000	0.321	0.000
	n	13	38	14
Pk-1	8	0.000	0.000	1.000
	11	1.000	1.000	0.000
Sdh	14	1.000	1.000	0.429
	18	0.000	0.000	0.571
	H	0.000	0.000	0.508
	n	33	10	14
Xdh	16	1.000	1.000	0.400
	19	0.000	0.000	0.600
	H	0.000	0.000	0.533
	n	43	15	5

*Expected heterozygosity, unbiased estimate (Nei, 1978).

Similarly, levels of DNAfp variability are extremely low in European bees, higher, but still low in comparison to other insects in the Indian ones. There are two main observations which require explanation, (i) the monomorphism in DNAfp profiles among European samples, and (ii) the DNAfp and allozyme differences between European and Indian bees. However, first we must address the question as to just how real these results are.

DNAfp is well known as a technique that permits differentiation between alternative genealogical hypotheses even among extremely closely related individuals (e.g. Jeffreys

Table 3. Summary statistics for allozyme variation in three populations of *L. marginatum*.

	Population		
	France	Greece	India
Mean sample size per locus	31.6	33.0	16.2
(SE)	1.6	1.7	0.7
Mean alleles per locus	1.0	1.1	1.2
Polymorphic loci (%)	2.1	6.4	17.0
Expected heterozygosity	0.008	0.021	0.060
(SE)	0.008	0.012	0.021

et al., 1985a). However, low levels of genetic variability have been reported using DNAfp methodologies for a wide range of organisms including nematodes (Uitterlinden *et al.*, 1989), frogs (Tegelstrom & Sjogren, 1990) and mammals (Gilbert *et al.*, 1990; Reeve *et al.*, 1990). Thus, our finding of little variation in *L. marginatum* is not without precedent. In addition, reasonably large amounts of genetic variability have been detected using the same protocol on a variety of insects including other bee species (Blanchetot, 1991a, b, c; A. Blanchetot and R. H. Gooding, unpublished data) and even in *L. comagenense* (A. Blanchetot and L. Packer, unpublished data) a close relative of *L. marginatum* (the two are in the same subgenus). The lack of variability in the European populations cannot, then, be attributed to our methodology as should be obvious from the variation we detect within the Indian sample and the differences observed between the Indian and European populations.

We conclude that the lack of DNAfp variability in *L. marginatum* from European and the DNAfp and allozyme differences between European and Indian samples are real biological phenomena requiring explanation. These unusual results could stem from molecular or ecological mechanisms or a combination of both. However, as nothing is known of the genome organization of *L. marginatum*, and because the level of DNAfp variability in this species is considerably lower than in the honey bee using an identical protocol, extrapolation of what is known of honey bee genome organization to *L. marginatum* is unwarranted.

We believe that the low levels of DNAfp variation in European samples of the *L. marginatum* species complex, and the differences between Indian and European samples, result from chronically low effective population sizes combined with a genetic bottleneck during the speciation event and the colonization of Europe.

Low levels of genetic variability and rapid rates of evolutionary change can result from small effective population size. Haplodiploid and sex linked genes have 75% of the effective population size of functionally diploid loci assuming a 1:1 sex ratio (Crozier, 1976). Consequently, such loci are expected to harbour lower levels of genetic variability. This may explain the reduced levels of allozyme and DNAfp variation in Hymenoptera (see Crespi, 1991, and herein) and lower amounts of DNAfp variability at sex-linked loci in birds (Longmire *et al.*, 1991). The following arguments suggest that there may be particularly small effective population sizes in the *L. marginatum* species complex.

Firstly, *L. marginatum* has a male-biased sex ratio in the reproductive brood (Quénu, 1959), thus further reducing the effective population size to around 65% of that which would obtain in a diploid organism with any sex ratio. Secondly, worker-produced males can further reduce effective population size (Owen & Owen, 1981). Thirdly, although it can be an abundant bee in some parts of Europe, the large numbers of workers found in mature nests means that the actual number of independent reproducing units is a small fraction (perhaps below 1%) of the number of individuals found foraging. Fourthly, in western Europe, at least, *L. marginatum* exists in small, isolated aggregations which are subject to catastrophic population crashes (Plateaux-Quénu, personal communication; Taylor, personal communication). In contrast, it is a more common species in the Mediterranean and thus more variability may be expected in the Greek than French populations. This was the pattern observed in the present study with both allozyme and DNAfp data. Lastly, males return to their natal nest at the end of the day in this species. This is unusual for bees and may be expected to constrain the distance between the nests of origin of mating partners. Thus, the effective size of the local population may be more closely restricted to that found in a single aggregation in *L. marginatum* than in most other bees. This may explain the low levels of genetic variation in this species but not the genetic differences between Indian and European samples to which we now turn.

Phylogeny and biogeography indicate that the *L. marginatum* complex probably originated near the eastern edge of its current geographical range: its closest relative, *L. salutatrix*, is only found in the foothills and valleys of the Himalaya mountains. Thus, this complex will have occurred in India for a longer period of time than in Europe. Indeed, it is probable that these bees did not disperse to Europe until after the retreat of cold weather after the last ice age, perhaps during the hypsithermal between 6000 and 8000 years ago: the unusual phenology and social biology of this species requires long summers as its present-day distribution suggests.

Both the almost complete absence of DNAfp variability in European bees and the large number of fixed allelic differences between Indian and European samples could be explained by a genetic bottleneck either during speciation and/or during the colonization of Europe. With chronically low population sizes and only small patches of habitat suitable for bees throughout arid Asia Minor, conditions were suitable for the fixation of mutant alleles in small populations as a result of genetic drift. A similar explanation was put forward to explain the low genetic identity between European and North American populations of another sweat bee *Halictus rûbicundus* (Packer & Owen, 1989) which is thought to have dispersed across the Bering Straight since the last ice age. Similar low levels of genetic variation at normally hypervariable loci have also been attributed to population bottlenecks (O'Brien *et al.*, 1983, 1985) and are generally noted to accompany founder speciation events (Barton, 1989) and colonization of new areas (Barrett & Shore, 1989). Our data from the *Lasioglos-*

sum marginatum complex adds another example to the body of literature.

Even if this scenario is correct, however, is it not true that DNAfp variation generation occurs rapidly enough for us to expect considerably less monomorphism? Levels of genetic variation for neutral variations depend upon both the mutation rate and the effective population size (Kimura, 1982). If DNAfp variation is generated by unequal crossing over during recombination, then a lower mutation rate is expected among haplodiploids and at sex-linked loci (see Longmire *et al.*, 1991, for an example of the latter in birds). Lower mutation rates as a result of male haploidy are not expected if replication slippage or gene conversion are the causes of DNAfp variation (Wolff *et al.*, 1991). Nonetheless, a lower mutation rate per unit time is expected in the *L. marginatum* complex in comparison to the other Hymenoptera for which DNAfp variation data are available simply because of the perennial nature of these bees. In contrast, leafcutting bees have at least one generation per year (Krunic, 1972) and honey bee colonies divide at least once a year even in cool temperate climates (Seeley, 1985). Thus, with its 5–6 year generation time, there may have been only 1000 generations since the dispersal of *L. marginatum* into Europe. This would have left little time for the generation of variability if the colonizing individuals were depauperate in DNAfp polymorphisms, as may be expected for reasons discussed.

The data presented here suggest that DNAfp may be a useful tool in insect population biology, particularly if used in conjunction with more traditional allozyme electrophoresis for which a broader range of background data are available for comparative purposes. Furthermore, our data suggest that the Hymenoptera may be particularly good model organisms with which to study population level phenomena by DNAfp.

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References

Barrett, S.H. and Shore, J. (1989) Isozyme variation in colonizing plants. In *Isozyme in Plant Biology* (Soltis, D.E. and Soltis, P.S. eds), pp. 106–126. Dioscorides Press, Portland, OR.

Barton, N.H. (1989) Founder effect speciation. In: *Speciation and its Consequences* (Otte, D. and Endler, J.A. eds), pp. 229–256. Sinauer Associates, Sunderland, MA.

Blanchetot, A. (1991a) Genetic variability of a satellite sequence in the Dipteran *Musca domestica*. In: *DNA Fingerprinting Approaches and Applications* (Burke, T., Dolf, G., Jeffreys, A.J. and Wolff, R. eds), pp. 106–112. Birkhauser-Verlag, Basel.

Blanchetot, A. (1991b) Genetic relatedness in honey bees as established by DNA fingerprinting. *J Hered* **82:** 391–396.

Blanchetot, A. (1991c) Intra-nest genetic relationships in the solitary leafcutting bee *Megachile rotundata* as analysed by DNA fingerprinting. *Genome* **35:** 681–688.

Brussard, P.F., Ehrlich, P.R., Murphy, D.D., Wilcox, D.A. and Wright, J. (1985) Genetic distances and the taxonomy of checkerspot butterflies (Nymphalidae: Nymphalinae). *J Kans Entomol Soc* **58:** 403–412.

Burke, T., Davies, N.B., Bruford, M.W. and Hatchewell, B.J. (1989) Parental care and mating behaviour of polyandrous dunnocks *Prunella modularis* related to paternity by DNA fingerprinting. *Nature* **338:** 249–251.

Burke, T., Dolf, G. Jeffreys, A.J. and Wolff, R. (1991) *DNA Fingerprinting: Approaches and Applications*. Birkhauser-Verlag, Basel.

Clayton, J.W. and Tretiak, D.N. (1972) Amine citrate tuffers for pH control in starch gel electrophoresis. *J Fish Res Bd Can* **29:** 1169–1172.

Crespi, B.J. (1991) Heterozygosity in the haplodiploid Thysanoptera. *Evolution* **45:** 458–464.

Crozier, R.H. (1976) Counter intuitive property of effective population size. *Nature* **262:** 384.

Feinberg, A.P. and Vogelstein, B.A. (1984) A technique for radiolabeling DNA restriction endonuclease fragments in high specific activity. *Anal Biochem* **137:** 266–267.

Gilbert, D.A., Lehman, N., O'Brien, S.J. and Wayne, R.K. (1990) Genetic fingerprinting reflects population differentiation in the California Channel Island Fox. *Nature* **344:** 764–766.

Grozdanic, S. (1966) Nova posmatranja na nekim vrstama roda *Halictus* (Hymenoptera). Prilog poznavanju filogenetskog postanka pcelinje porodice. *Inst Biol Istraz Zborn* **10:** 29.

Jeffreys, A.J., Brookfield, J.F.Y. and Semeonoff, R. (1985a) Positive identification of an immigration test-case using human DNA fingerprints. *Nature* **317:** 818–819.

Jeffreys, A.J. and Morton, D.B. (1987) DNA fingerprints of dogs and cats. *Anim Genet* **18:** 1–15.

Jeffreys, A.J., Wilson, V. and Thein, S.L. (1985b) Individual specific fingerprints of human DNA. *Nature* **316:** 76–79.

Kimura, M. (1982) *The Neutral Theory of Molecular Evolution*. Cambridge University Press.

Krunic, M.D. (1972) Voltinism in *Megachile rotundata* (Megachilidae: Hymenoptera) in southern Alberta. *Can Ent* **104:** 165–167.

Longmire, J.L., Ambrose, R.E., Brown, N.C., Cade, T.J., Maechtle, T.L., Seegar, W.S., Ward, F.P. and White, C.M. (1991) Use of sex-linked minisatellite fragments to investigate genetic differentiation and migration of North American populations of the peregrine falcon (*Falco peregrinus*) In: *DNA Fingerprinting: Approaches and Applications* (Burke, T., Dolf, G. Jeffreys, A.J. and Wolff, R. eds), pp. 217–229. Birkhauser-Verlag, Basel.

Lynch, M. (1990) The similarity index and DNA fingerprinting. *Mol Biol Evol* **7:** 478–484.

Lynch, M. (1991) Analysis of population genetic structure by DNA fingerprinting. In: *DNA Fingerprinting: Approaches and Appli-*

cations (Burke, T., Dolf, G., Jeffreys, A.J., and Wolff, R. eds), pp. 113–126. Birkhauser-Verlag, Basel.

Meng, A., Carter, R.E. and Parkin, D.T. (1990) The variability of DNA fingerprint in the three species of swan. *Heredity* **64:** 73–80.

Nei, M. (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89:** 583–590.

O'Brien, S.J., Roelke, M.E., Marker, L., Newman, A., Winkler, C.A., Meltzer, D., Colly, L., Evermann, J.F., Bush, M. and Wildt, D.E. (1985) Genetic basis for species vulnerability in the cheetah. *Science* **227:** 1428–1434.

O'Brien, S.J., Wildt, D.E., Goldman, D., Merril, C.R. and Bush, M. (1983) The cheetah is depauperate in biochemical genetic variation. *Science* **221:** 459–462.

Owen, R.E. and Owen, A.R.G. (1981) Effective population size in social Hymenoptera with worker-produced males. *Heredity* **63:** 59–65.

Packer, L. (1991) The evolution of social behavior and nest architecture in sweat bees of the sugenus *Evylaeus* (Hymenoptera: Halictidae): a phylogenetic approach. *Behav Ecol Sociobiol* **29:** 153–160.

Packer, L. and Owen, R.E. (1989) Allozyme variation in *Halictus rubicundus* (Christ): a primitively social Halictine bee (Hymenoptera: Halictidae). *Can Ent* **121:** 1049–1058.

Packer, L. and Owen, R.E. (1990) Allozyme variation, linkage desequilibrium and diploid male production in a primitively social bee *Augochlorella striata* (Hymenoptera: Halictidae). *Heredity* **65:** 241–248.

Packer, L. and Owen, R.E. (1992) Variable enzyme systems in the Hymenoptera. *Biochem Syst Ecol* **20:** 1–7.

Qavi, H. and Kit, S. (1980) Electrophoretic patterns of aminoacylase (Acy-1) isozymes in vertebrate cells and histochemical procedure for detecting Acy-1 activity. *Biochem Genet* **18:** 669–679.

Quénu, C. (1959) Un nouveau type de société d'insectes, *Halictus marginatus* Brullé. *Ann Biol* **35:** 235–444.

Reeve, H.K., Westneat, D.F., Noon, W.A., Sherman, P.W. and Aquadro, C.F. (1990) DNA fingerprinting reveals high levels of inbreeding in colonies of the eusocial naked mole rat. *Proc Natl Acad Sci USA* **87:** 2496–2500.

Ridgway, G.L., Sherburne, S.U. and Lewis, R.D. (1970) Polymorphism in the esterase of Atlantic herring. *Trans Am Fish Soc* **99:** 147–151.

Ryskov, A.P., Jincharadze, A.G., Prosnyak, M.I. Ivanov, P.L. and Limborska, S.A. (1988) M13 phage DNA as a universal marker for DNA fingerprinting of animals, plants and microorganisms. *FEBS Lett* **233:** 388–392.

Seeley, T. (1985) *Honeybee Ecology.* Princeton University Press, New Jersey.

Tegelstrom, H. and Sjogren, P. (1990) Monomorphic DNA-fingerprints in northern pool frogs (*Rana lessonae*). *Fingerprint News* **2:** 7–9.

Shaw, C.R. and Prasad, R. (1970) Starch gel electrophoresis of enzymes: a compilation of recipes. *Biochem Gen* **4:** 291–320.

Swofford, D.L. and Selander, R.B. (1989) BIOSYS-1: A computer program for the analysis of allelic variation in population genetics and biochemical systematics. I11. *Nat Hist Surv* p. 43.

Uitterlinden, A.G., Slagboom, P.E., Johnson, T.R. and Vijg, J. (1989) The *Caenorhabditis elegans* genome contains monomorphic minisatellites and simple sequences. *Nucl Acids Res* **17:** 9527–9530.

Vassart, G., Georges, M., Monsieur, R., Brocas, H., Lequarré, A.S. and Christophe, D.A. (1987) Sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. *Science* **235:** 683–684.

Westneat, D.F., Noon, W.A., Reeve, H.K. and Aquadro, C.F. (1988) Improved hybridisation conditions for DNA fingerprint probed with M13. *Nucl Acids Res* **16:** 4161.