Molecular Ecology (2001) **10**, 1423–1432

Hierarchical genetic structure of the introduced wasp *Vespula germanica* in Australia

MICHAEL A. D. GOODISMAN,*‡ ROBERT W. MATTHEWS† and ROSS H. CROZIER*
*Department of Genetics, La Trobe University, Bundoora, VIC, 3083, Australia and Department of Zoology and Tropical Ecology, James Cook University, Townsville, QLD, 4811, Australia, †Department of Entomology, University of Georgia, Athens, GA, 30602, USA

Abstract

The wasp *Vespula germanica* is a highly successful invasive pest. This study examined the population genetic structure of *V. germanica* in its introduced range in Australia. We sampled 1320 workers and 376 males from 141 nests obtained from three widely separated geographical areas on the Australian mainland and one on the island of Tasmania. The genotypes of all wasps were assayed at three polymorphic DNA microsatellite markers. Our analyses uncovered significant allelic differentiation among all four *V. germanica* populations. Pairwise estimates of genetic divergence between populations agreed with the results of a model-based clustering algorithm which indicated that the Tasmanian population was particularly distinct from the other populations. Within-population analyses revealed that genetic similarity declined with spatial distance, indicating that wasps from nests separated by more than ~25 km belonged to separate mating pools. We suggest that the observed genetic patterns resulted from frequent bottlenecks experienced by the *V. germanica* populations during their colonization of Australia.

Keywords: DNA microsatellite, introduced species, migration, social insects, yellowjacket wasp

Received 7 October 2000; revision received 8 January 2001; accepted 8 January 2001

Introduction

The organization of natural ecosystems may be disrupted by the invasion of foreign species (Mooney & Drake 1986; Drake *et al*. 1989; Williamson 1996). Social insects are a particularly notorious group of invaders. Various introduced termites, ants and wasps have caused substantial environmental and economic harm in many of their introduced habitats (Vinson 1986; Williams 1994; Moller 1996). The European wasp or German yellowjacket, *Vespula germanica*, is an especially successful and destructive social insect in its introduced range. Originating from Europe, Asia and North Africa, *V. germanica* now enjoys a nearly circumglobal distribution, having established populations in North America, South America, South Africa, New Zealand and Australia (Spradbery 1973; Akre & MacDonald 1986; Clapperton *et al*. 1989). *Vespula* wasps cause considerable harm to indigenous wildlife because they are voracious polyphagous predators on native arthropods and outcompete other animals for food (e.g. Moller & Tilley 1989; Beggs & Wilson 1991; Fordham 1991; Harris 1991; Beggs 2000).

V. germanica was first discovered in Australia on the island of Tasmania in 1959 (*V. germanica* colonization of Australia reviewed by Crosland 1991; Spradbery & Maywald 1992). However, it was not recorded on the Australian mainland until 15 years later. By then it was found in Adelaide, Melbourne, Perth and Sydney. Subsequently *V. germanica* established itself as a major pest throughout the south-east of the country. Estimates of the natural dispersal rate of *V. germanica* indicate that the wasp can spread no more than ~1000 m per year (Thomas 1960; Edwards 1980; Crosland 1991). This relatively short dispersal distance cannot account for the rapid appearance of *V. germanica* in Australian cities separated by hundreds of kilometres. Rather, the movement of *V. germanica* over such long distances probably results from the accidental transport of hibernating queens (Thomas 1960; Crosland 1991; Spradbery & Maywald 1992). Such human-aided dispersal normally involves the movement of relatively few queens

Correspondence: Michael A. D. Goodisman. ‡Present address: c/o Dr Wells Lab-Department of Biochemistry, Biological Sciences West, PO Box 210088, University of Arizona, Tucson, AZ 85721-0088, USA. Fax: 520-621-9288; E-mail: goodisma@email.arizona.edu

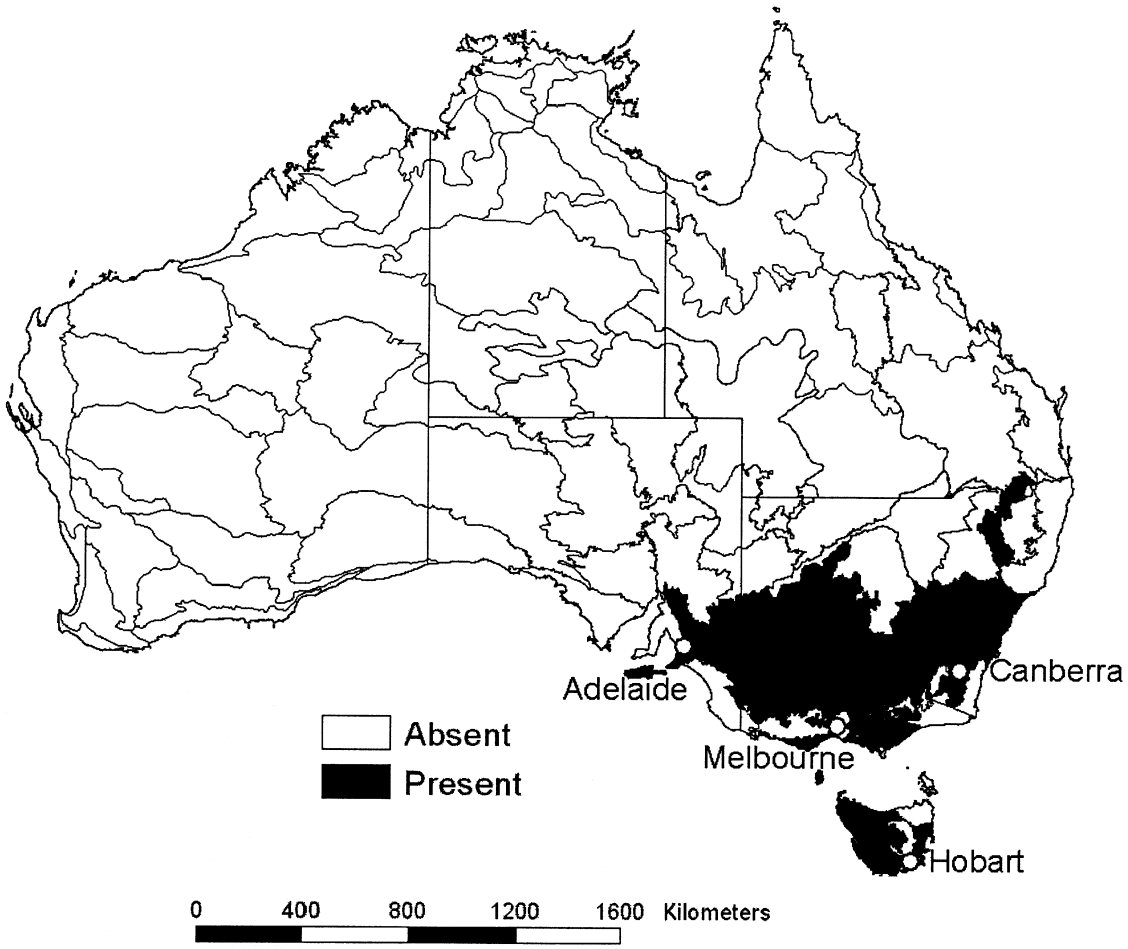

Fig. 1 Current distribution of *Vespula germanica* in Australia by interim biogeographic region (modified and reprinted with permission from Clarke *et al*. 2001) and locations of four populations sampled in this study.

(Crosland 1991), and thus causes bottlenecks during the establishment of wasp populations.

The purpose of this study is to investigate the population genetic structure of the *V. germanica* in Australia using DNA microsatellite markers. The manner in which *V. germanica* has colonized Australia leads us to predict that introduced populations will display a particular pattern of hierarchical genetic structure. Specifically, if *V. germanica* populations underwent bottlenecks when they were founded, then they should also have been subjected to strong episodes of genetic drift (Chakraborty & Nei 1977). Genetic drift, in turn, would have resulted in significant differentiation among independently founded populations. We do not anticipate substantial structure within populations because we expect that the flight capabilities of queens would be sufficient to homogenize allele frequencies on a local level. Overall, this study should help elucidate the manner in which social insects establish populations when introduced into new habitats.

Materials and methods

Sampling

Vespula germanica wasps were collected from annual colonies in the austral summer and autumn of 1998/99. Samples were obtained from populations around the cities of Adelaide, Canberra and Melbourne, which are found on the Australian mainland, and Hobart, which is located on the Australian island of Tasmania (Fig. 1). Wasps were netted individually, collected from excavated nests, or captured in Malaise traps. All wasps captured away from their nests (e.g. those captured in Malaise traps) were assumed to originate from different nests and thus represent genetically independent samples. Once caught, specimens were immediately frozen or placed in 95% ethanol for subsequent DNA analysis. The samples consisted mostly of workers; however, males were also obtained opportunistically.

Laboratory methods

We assayed the genotypes of all individuals at three polymorphic microsatellite markers, Rufa 5, Rufa 18 and Rufa 19, originally developed for *V. rufa* (P. Thorén, unpublished results). Genomic DNA for microsatellite analysis was extracted from single *V. germanica* legs using a variation of the Chelex® protocol (Walsh *et al*. 1991) as described by Crozier *et al*. (1999). The loci were polymerase chain reaction (PCR) -amplified using either fluorescently or radioactively labelled PCR primers.

To visualize microsatellites using fluorescent techniques, the forward primers of Rufa 5, 18 and 19 were first labelled with TET, FAM and TET dyes, respectively. PCRs were conducted in a final volume of 15 μL containing 3 μL genomic DNA and 0.75 U *Taq* DNA polymerase (Promega), and a final concentration of 200 μM dNTPs, 0.5 μM of each of the forward and reverse PCR primers and 1× Promega Buffer (with 1.5 mM $MgCl_2$). PCR products were then electrophoresed and scored on an ABI Prism 377 DNA Sequencer.

To visualize microsatellites using radioactivity, the forward PCR primer for amplifying each locus was first end-labelled with [γ^{33}P]ATP. PCRs were then carried out in a final volume of 10 μL containing 2 μL genomic DNA, 5 μg bovine serum albumin (New England Biolabs) and 0.4 U *Taq* DNA polymerase, and a final concentration of 167 μM dNTPs, 0.4 μM of unlabelled reverse primer, 0.1 μM of unlabelled forward primer, 0.03 μM of radioactively end-labelled forward primer and 1× Promega Buffer (with 1.5 mM $MgCl_2$). PCR products were run on 5% denaturing polyacrylamide gels at a constant power of 65 W for 3–4 h and then scored after exposing the dried gel to film (Kodak or Fuji) overnight. For both the fluorescent and radioactive methods, the PCR cycling profiles for the three markers began with an initial denaturation at 94 °C for 2 min, and then proceeded with 30 or 35 cycles of 93 °C for 30 s, 55 °C (Rufa 5 and 19) or 52 °C (Rufa 18) for 30 s, and 72 °C for 30 s, followed by a final extension of 72 °C for 10 min.

The wasps were also screened for restriction fragment-length polymorphisms (RFLPs) at a single PCR-amplified portion of the mitochondrial DNA (mtDNA). The ~1500 bp fragment, which spanned parts of the COI and COII genes, as well as an intergenic spacer region, was amplified using the primers C1-J-2183 and C2-N-3661 (Simon *et al*. 1994). PCRs were carried out in a final volume of 50 μL containing 5 μL genomic DNA, 0.3 U *Taq* DNA polymerase and a final concentration of 200 μM dNTPs, 0.5 μM of each primer and 1× Promega Buffer (with 1.5 mM $MgCl_2$). The PCR cycling profile began with an initial denaturation at 93 °C for 3 min, and then proceeded with 35 cycles of 93 °C for 30 s, 55 °C for 30 s and 72 °C for 90 s, followed by a final extension of 72 °C for 10 min.

The PCR-amplified fragment of each wasp was individually digested with the restriction enzymes *Acc*I, *Afl*II, *Alu*I, *Asc*I, *Bsp*DI, *Bsr*GI, *Dde*I, *Dra*I, *Eco*RV, *Hin*fI, *Mse*I, *Ssp*I, *Tsp*509I and *Xba*I according to supplier recommendations. The fragments were electrophoresed on a 3.5% agarose gel containing ethidium bromide and visualized with UV light.

Statistical analyses

Allele frequencies at the three microsatellite markers within each of the four populations were estimated using the program RELATEDNESS 4.2, with nests weighted equally (Queller & Goodnight 1989). Nei's (1987) unbiased estimate of gene diversity,

$$h_{jk} = 2n\,(1 - \Sigma_i\, p_{ijk}^2)/(2n - 1) \qquad (1)$$

where n is the number of diploid individuals sampled and p_{ijk} is the frequency of allele i at locus j (for $j = 1 - 3$ where Rufa 5, Rufa 18 and Rufa 19 are loci 1, 2 and 3) in population k (for $k = 1 - 4$ where Adelaide, Canberra, Hobart and Melbourne are populations 1, 2, 3 and 4), was used as a measure of variability for each marker in each population.

We investigated if the levels of genetic variation differed across southeastern Australia by testing if the mean gene diversity of all three microsatellite markers,

$$H_k = (\Sigma_j\, h_{jk})/3$$

varied among the four populations. We conducted these comparisons using two different population sample sizes to account for instances where multiple wasps were collected per nest. First, we conservatively took the sample size within each population to equal the number of nests from which wasps were obtained (N_k). Second, we considered a less restrictive sample size, which incorporated information on the total number of haploid genomes detected in a given population. This method relies on the fact that annual *V. germanica* colonies are headed by a single, multiply mated queen (M. Goodisman, R. Matthews and R. Crozier, unpublished results). Therefore, genetic information on both the queen and her multiple male mates is obtained when more than a single wasp is sampled per nest. The effective number of diploid breeding individuals sampled in population k can be given by

$$n_k = (2 + \bar{m}_k)\, N_k/2 \qquad (2)$$

Here, $\bar{m}_k$ is the mean of the minimum number of male mates in population k; estimates of $\bar{m}_k$ for these populations were obtained from a previous study (M. Goodisman, R. Matthews and R. Crozier, unpublished results). In eqn 2, the term $2 + \bar{m}_k$ represents the average number of chromosomes sampled per nest (two for the queen and $\bar{m}_k$ for her

male mates). This value is divided by two to yield the effective diploid sample size per nest and then multiplied by N_k to yield the total sample size for the population.

To decide if populations differed significantly in their variability we multiplied the average gene diversity in population k, H_k, and the average gene identity, $1 - H_k$, by the sample size (either N_k or n_k), which provided the expected number of heterozygotes and homozygotes in each population. These values were then rounded to the nearest integer and the proportion of heterozygotes to homozygotes was compared among populations using a G-test of independence (Sokal & Rohlf 1995).

We used reduced data sets including only a single individual per nest for most subsequent population analyses, because nestmates are related and, consequently, cannot be considered independently of one another. To avoid the problems caused by this genetic nonindependence, we used a resampling technique that yielded unbiased measures of population genetic structure (e.g. Ross & Keller 1995b). Briefly, a computer program was written to select randomly a single individual's multilocus genotype from each nest, yielding a new data set with the number of individuals sampled equal to the number of nests. This procedure was repeated 25 times to produce 25 such data sets. Each of these resampled data sets was then used to calculate the population statistic or probability value of interest, and the median of the 25 values was taken as the unbiased estimate. Unless stated otherwise, the program GENEPOP 3.1c (Raymond & Rousset 1995) was used in conjunction with this resampling procedure in subsequent calculations.

The significance of genotypic disequilibrium between microsatellite markers, deviations from Hardy–Weinberg equilibrium and allelic differentiation between populations was calculated using a probability test. Bonferroni corrections were applied when multiple tests were performed (Rice 1989) and probability values for all loci combined or all populations combined were determined using Fisher's method of combined probability (Sokal & Rohlf 1995).

The genetic structure within and among populations was quantified using two different methods. First, we estimated Wright's hierarchical F-statistics, F_{IS}, F_{ST} and F_{IT}, using Weir & Cockerham's (1984) f, θ and F, respectively. Second, we measured population structure using the metrics ρ_{IS}, ρ_{ST} and ρ_{IT}, which incorporate information on the stepwise mutation process believed to operate in microsatellite loci in the estimation of hierarchical genetic structure (Rousset 1996). Estimates of θ and ρ_{ST} were also calculated for all pairs of populations.

We used the model-based clustering algorithm of Pritchard *et al*. (2000) as implemented by the program STRUCTURE to investigate further the patterns of differentiation in the introduced *V. germanica* populations. The method accounts for Hardy–Weinberg and linkage disequilibrium among all samples and attempts to group individuals into populations that are in equilibrium. Thus, for these calculations our data set consisted of a single randomly selected diploid (i.e. worker) wasp per nest. The procedure was used to group wasps into K theoretical populations, where K varied from one to nine. The probability of the number of populations equalling K was calculated from the estimated log probability of the data. The method also yielded the admixture proportions, $q^{(i)}$, which represent the proportional membership of the genome of wasp i in each of the K populations.

Finally, we tested for evidence of genetic isolation by distance within the populations of Canberra and Melbourne, where the specific collecting locations of nests had been recorded. We first calculated Nei's (1978) genetic distances between all pairs of nests using the program GENETIC DATA ANALYSIS (Lewis & Zaykin 2000). Individual wasps captured away from nests were omitted in these analyses and the resampling procedure described above was not applied. Spearman's rank order correlation coefficient (r_S) was used to measure the association between the genetic distances between nests and the geographical distances that separated them. Significance of the correlation between geographical and genetic distance was assessed with a Mantel test, which was performed by the program GENEPOP 3.1c. The relationship was deemed to be significant if the observed correlation was greater than 5% of the 10 000 randomly generated values.

Results

Workers and males were sampled from all populations except Hobart, where only workers were collected (Table 1). All wasps from Adelaide and Canberra, and all but seven wasps that were individually netted in Melbourne, were collected directly from nests. All of the Hobart samples were captured in Malaise traps. The gene diversities for each marker in the four populations calculated using eqn 1 revealed moderate levels of genetic variation (Table 2). However, our screen for RFLPs at the PCR-amplified mtDNA fragment revealed no variation; all individuals possessed the same mitochondrial haplotype.

Table 1 Number of nests (N), workers (n_w) and males (n_m) sampled from four introduced populations of *Vespula germanica* in Australia

Population	N	n_w	n_m
Adelaide	11	66	58
Canberra	36	840	236
Hobart	76	76	0
Melbourne	18	338	82
Total	141	1320	376

Table 2 Allele frequencies at three microsatellite loci in four populations of introduced *Vespula germanica* from Australia. Gene diversities for each locus (*h*) calculated assuming sample size equals the number of nests from which wasps were collected (see text). Allele names correspond to the size of the product amplified by the locus-specific PCR primers

Locus and population	Allele							*h*
Rufa 5	143	145	147	149	155	177		
Adelaide	0.074	0.568	0.000	0.017	0.000	0.341		0.565
Canberra	0.000	0.386	0.000	0.614	0.000	0.000		0.477
Hobart	0.329	0.664	0.007	0.000	0.000	0.000		0.454
Melbourne	0.000	0.690	0.000	0.238	0.001	0.071		0.467
Rufa 18	187	195	197	199	201	203	205	
Adelaide	0.000	0.006	0.047	0.222	0.138	0.564	0.023	0.621
Canberra	0.000	0.000	0.000	0.305	0.067	0.628	0.000	0.511
Hobart	0.000	0.007	0.014	0.760	0.014	0.205	0.000	0.382
Melbourne	0.017	0.042	0.154	0.243	0.230	0.314	0.000	0.771
Rufa 19	200	202	204	206	208	210	212	
Adelaide	0.216	0.160	0.274	0.201	0.038	0.000	0.110	0.813
Canberra	0.162	0.756	0.052	0.020	0.004	0.005	0.000	0.402
Hobart	0.743	0.250	0.007	0.000	0.000	0.000	0.000	0.388
Melbourne	0.228	0.393	0.054	0.072	0.028	0.000	0.224	0.742

From eqn 1, we found that the mean gene diversities for the three microsatellite markers, H_k, in Adelaide, Canberra, Hobart and Melbourne were 0.69, 0.47, 0.41 and 0.67, respectively, given that the sample size was equal to the number of nests from which wasps were collected (Table 1). Therefore, the expected numbers of heterozygotes : homozygotes in the four populations were 8 : 3, 17 : 19, 31 : 45 and 12 : 6; these proportions did not differ significantly among populations ($G_3 = 6.92$, $P = 0.07$). The effective numbers of diploid individuals, n_k, calculated from eqn 2 were 29.15, 78.66, 76.00 and 48.24 in Adelaide, Canberra, Hobart and Melbourne, respectively. Consequently, H_k values were 0.67, 0.46, 0.41 and 0.66 in the four populations given these latter sample sizes. The expected ratios of heterozygotes : homozygotes, 19 : 10, 36 : 42, 31 : 45 and 32 : 17, now differed significantly among populations ($G_3 = 10.49$, $P = 0.015$). Thus, the results of both tests considered together provided some evidence that populations differed in their variability.

We found no evidence of linkage disequilibrium between any of the loci in the populations ($P > 0.2$ for all pairs of loci) and therefore considered each microsatellite marker as providing independent genetic information. Probability tests did not reveal significant deviations from Hardy–Weinberg equilibrium for each locus when the locus-specific probability values in each population were combined ($P > 0.3$ for all loci). Thus, our data showed no indication of a high frequency null allele at any of the microsatellite markers. If present, such an allele would be expected to lead to excess apparent homozygosity within populations and, consequently, to significant deviations from Hardy–Weinberg proportions. Probability tests also failed to uncover deviations from Hardy–Weinberg equilibrium within each population for all loci combined ($P > 0.3$ in all populations) suggesting that mating occurred at random within populations (but see below). This result also indicated that wasps captured in Malaise traps in Hobart probably originated from many different families. If the Hobart samples were obtained from few families, then we would have expected a significant excess of heterozygotes relative to Hardy–Weinberg equilibrium (Michod 1980).

The four populations differed substantially in allele frequencies (Table 2). The null hypothesis for allelic homogeneity between all pairs of populations was strongly rejected even after sequential Bonferroni corrections were applied ($P = 0.02$ for the Adelaide–Melbourne comparison, $P < 0.0001$ for all other pairwise comparisons). Not surprisingly, the probability test for all populations combined also yielded a highly significant *P*-value ($P < 0.0001$), indicating significant genetic differentiation among populations.

The overall estimates of Wright's *F*-statistics, $f = -0.01$, $\theta = 0.28$ and $F = 0.27$, and the microsatellite-specific ρ-statistics, $\rho_{IS} = -0.06$, $\rho_{ST} = 0.30$ and $\rho_{IT} = 0.26$, revealed similar patterns and agreed with expectations arising from the probability tests for Hardy–Weinberg equilibrium and allelic homogeneity among populations. Estimates of *f* and ρ_{IS} were both small and negative, providing little evidence for nonrandom mating within populations. In contrast, the metrics of population differentiation, θ and ρ_{ST}, were sizeable, leading to high overall coefficients of population genetic structure, *F* and ρ_{IT}. Estimates of θ and ρ_{ST} for the pairwise comparisons among populations (Table 3) also revealed substantial genetic differentiation between populations, with Hobart displaying particularly strong divergence from the other populations.

Table 3 Pairwise estimates of genetic differentiation between four introduced *Vespula germanica* populations in Australia. Values above the main diagonal are estimates of θ and those below the diagonal are estimates of ρ_{ST}. All pairs of populations differed significantly ($P < 0.05$) as judged by a probability test for allelic differentiation

	Adelaide	Canberra	Hobart	Melbourne
Adelaide		0.21	0.27	0.06
Canberra	0.21		0.36	0.10
Hobart	0.64	0.55		0.24
Melbourne	0.01	0.07	0.41	

Results of the clustering algorithm of individual wasps revealed that the most likely estimate of the number of populations, *K*, that could account for the observed genotypes was three ($P = 0.999$). All other values of *K* were deemed highly improbable ($P < 0.0001$). Figure 2 shows the values of $q^{(i)}$, the proportional membership of wasp *i* in each of the three theoretical populations under the 'most likely' model. This figure further illustrates the patterns of genetic structure among populations in this study. The wasps from Hobart have largely maintained their distinctiveness. However, the samples from Melbourne have been alternately lumped with those from Adelaide or Canberra, thus reducing the number of populations needed to explain the data.

Finally, we examined the data for evidence of isolation by distance within individual populations. The relationship between Nei's genetic distance and geographical distance in Canberra and Melbourne is shown in Fig. 3. The mean geographical distances between all pairs of nests in Canberra and Melbourne were 5.56 km and 80.20 km, respectively. The overall patterns in the two populations differed considerably, in part because of the scale at which nests were sampled. In Canberra, all nests were relatively close to one another and showed substantial genetic similarity. This relationship was reflected by a low and non-significant correlation between genetic and geographical

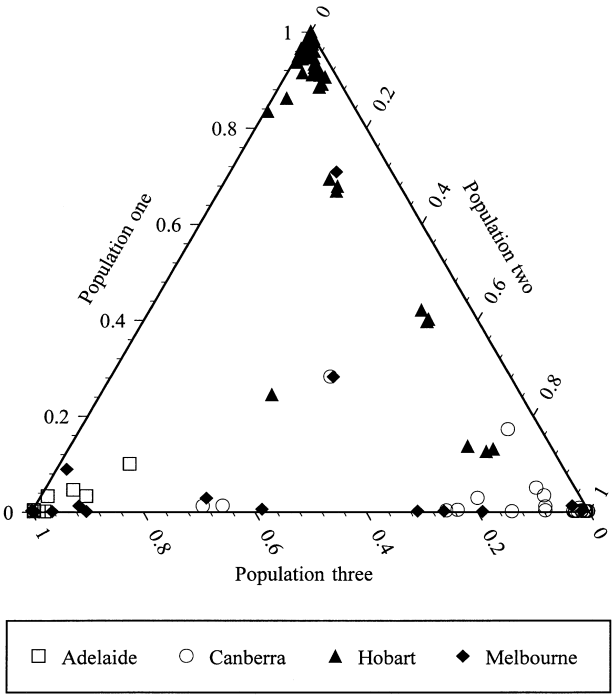

Fig. 2 Proportional membership of introduced *Vespula germanica* wasps in the three theoretical populations (1, 2 and 3) deemed most likely to account for data under a model-based clustering algorithm (see text for details). A point lying in one of the corners of the diagram represents an individual judged as belonging almost exclusively to one of the three populations.

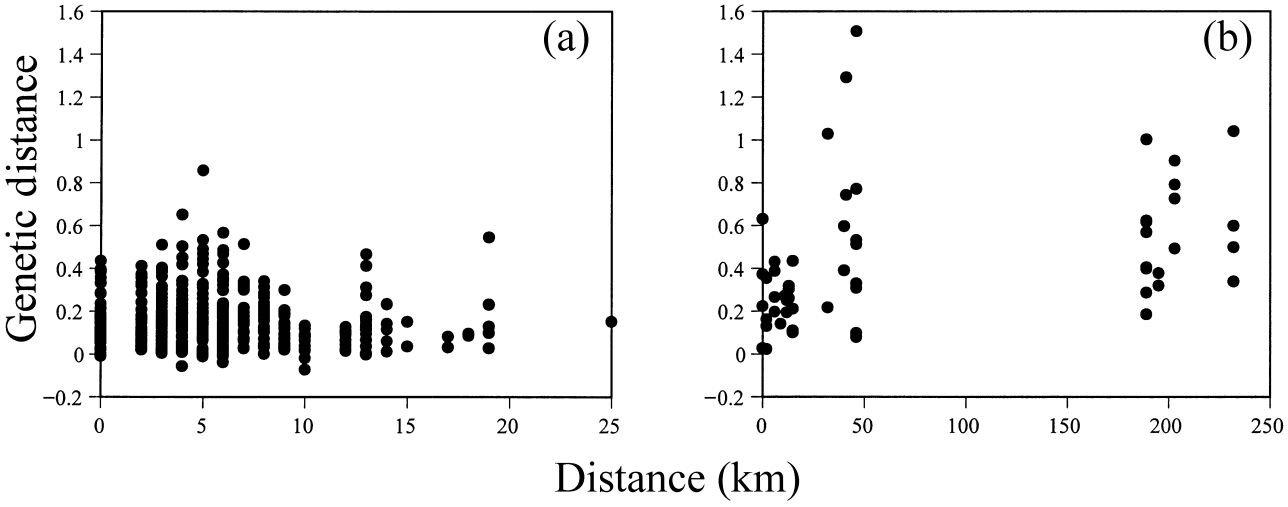

Fig. 3 Relationship between genetic distance, as measured by Nei's *D*, and geographical distance between pairs of introduced *Vespula germanica* nests in (a) Canberra and (b) Melbourne. Note difference in scale of axes.

distance ($r_S = -0.058$, $P = 0.74$). In contrast, sampling in Melbourne took place over a much wider area. Nests less than 25 km from each other were genetically similar (Fig. 3). However, nests separated by greater distances frequently displayed substantial genetic differentiation. Thus, the overall pattern in Melbourne resulted in a significant association between genetic and geographical distance ($r^S = 0.51$, $P = 0.0096$).

Discussion

Genetic structure among Australian Vespula germanica *populations*

The central finding from this study is that the introduced *Vespula germanica* displayed significant genetic structure among distantly separated populations in Australia. Moreover, many of the pairwise estimates of θ and ρ_{ST} between populations were substantial (Table 3). We note, however, that neither of these statistics should be used to estimate the time of divergence among populations nor the number of migrants exchanged between populations, because the processes that generated genetic differentiation in Australian *V. germanica* probably do not correspond to those assumed in making such calculations (Goldstein *et al*. 1995; Slatkin 1995). Rather, the observed genetic patterns reflect the way in which *V. germanica* colonized Australia.

The clustering algorithm of Pritchard *et al*. (2000) was used to determine the most probable number of populations that could explain the patterns of Hardy–Weinberg and linkage disequilibrium when all wasps were considered together. This analysis indicated that three, rather than four, populations could best explain the data. Wasps from Melbourne apparently could be considered as Adelaide-like or Canberra-like. These results were generally consistent with the pairwise estimates of θ and ρ_{ST}, which were lower for the Melbourne-Adelaide and Melbourne-Canberra comparisons than for the other population comparisons (Table 3). These analyses suggest that wasps from Melbourne may have founded the populations of Adelaide and Canberra. Further support for this hypothesis arises from the fact that the Adelaide and Canberra populations tended to possess subsets of the alleles present in the Melbourne population.

The overall patterns of differentiation also indicated that the island population of Hobart differed more from any of the mainland populations than the mainland populations differed from each other. This result suggests that colonization of the mainland may not have occurred via Tasmania and may instead represent a separate introduction from elsewhere. Addressing the origin of the *V. germanica* in Australia would require data on the genetic composition of populations from outside Australia. Knowledge of the relationship of Australian *V. germanica* populations to those in New Zealand, the closest country to Australia where *V. germanica* occurs, would be particularly instructive. Genetic comparisons to native Palearctic populations would also help elucidate the origin of Australian *V. germanica*.

The among-population genetic structure uncovered in this study is consistent with the hypothesis that the establishment of Australian populations involved genetic bottlenecks. The colonization history of *V. germanica* suggests that relatively few queens found introduced populations. Moreover, once introduced to a new area, *V. germanica* populations often remain small for a few years before undergoing an exponential increase (Crosland 1991). Thus, bottlenecks may accompany the invasion of new habitats, resulting in strong genetic drift and, consequently, in genetic differentiation among populations (Chakraborty & Nei 1977). This founder effect may be further exacerbated by the frequent, but largely unexplained, yearly fluctuations in the size of *V. germanica* populations (Archer 1980; Horwood *et al*. 1993; Harris & Beggs 1995). These swings result in low, long-term effective population sizes (Hartl & Clark 1989), making genetic drift an even greater factor leading to a high variance in allele frequencies among introduced populations.

The bottlenecks posited to occur in introduced *V. germanica* should affect the levels of genetic variation within populations. We found some evidence of significant differences in the variability among Australian populations as measured by the average gene diversity. This result suggests that the effective population sizes of the wasps have differed. Comparisons of the estimates of variability at the microsatellite loci and the invariant PCR-amplified mtDNA fragment in Australian populations to those in native populations would further elucidate the colonization history of *V. germanica*. We predict that measures of variability in native populations would be substantially greater than in Australia, as expected if *V. germanica* passed through bottlenecks during its introduction.

Genetic structure within Australian V. germanica *populations*

In addition to documenting significant differentiation between introduced *V. germanica* populations, we detected evidence of genetic isolation by distance within the population of Melbourne, but not Canberra. The contrasting findings from the two populations are probably related to the scales at which sampling took place. In Melbourne, nests were sampled over a relatively large geographical area, spanning distances up to ~225 km (Fig. 3). However, in Canberra sampled nests were much closer, at most ~25 km. The combined data from Canberra and Melbourne suggest that no significant relationship between genetic similarity and geographical distance exists between nests separated by less than ~25 km. However, wasps from nests

separated by greater distances may belong to separate mating pools.

The finding of significant isolation by distance in Melbourne may appear incompatible with the nonsignificant deviation from Hardy–Weinberg equilibrium found in that population. That is, we would predict that genetic differentiation within populations would lead to heterozygote deficiency as expected under the Wahlund effect (Hartl & Clark 1989). However, this result is probably related to the fact that a few outlier nests contributed disproportionately to the test of isolation by distance. These nests were involved in many of the pairwise distance comparisons. However, they did not affect the Hardy–Weinberg tests to as high a degree, because the resampling protocol we used in those calculations only incorporated information from a single individual per nest (see Methods).

The discovery of genetic isolation by distance within an introduced population of *V. germanica* was not expected. We predicted that allele frequencies within populations would be fairly homogeneous, because *Vespula* wasps possess relatively strong flight capabilities (Edwards 1980; Matsuura & Yamane 1984; Ross & Carpenter 1991; Moller 1996). There are at least two nonexclusive explanations that could result in this pattern. First, the population in Melbourne may actually result from two or more recent introductions of genetically differentiated founders. Under this scenario, we might expect the allele frequencies throughout the Melbourne area to homogenize given sufficient time. A second possibility is that the flight capabilities of the European wasp are insufficient to produce panmixia among individuals from nests separated by more than 25 km. Under this scenario, local mating and philopatry in conjunction with genetic drift would lead to genetic differentiation on relatively small scales. Support for this latter hypothesis arises from examination of the distribution of pairwise genetic distances between nests, which were found not to differ significantly from a normal distribution (data not shown), as expected under gradual isolation by distance.

Genetic structure in introduced social insects

Genetic structure above the level of the colony has been investigated in only a few other introduced social insects. One of the best-studied examples is that of the honeybee *Apis mellifera*. *Apis mellifera* is native to Europe, Asia and Northern Africa, but has been introduced to North America, South America and Australia (Michener 1974). Population genetic studies of *A. mellifera* in South America and the United States have detected substantial differentiation using nuclear and mitochondrial markers (Lobo *et al*. 1989; Schiff *et al*. 1994; Lobo 1995). In Australia, a study using allozymes and mtDNA found that honeybees across Tasmania displayed significant genetic structure, although it was suggested that selection and hybridization may have played a role in determining the observed population genetic patterns (Oldroyd *et al*. 1995). Overall, studies of *A. mellifera* support the view that significant differentiation may exist in introduced social insects. However, the genetic structure of introduced *A. mellifera* is likely to be directly or indirectly affected by gene flow from managed bee populations. Moreover, movement of honeybees is often intentional. Therefore, it may be inappropriate to compare the population genetic patterns of introduced *A. mellifera* to those in introduced *V. germanica*.

In contrast, the history and invasion biology of the red imported fire ant, *Solenopsis invicta*, and the Argentine ant, *Linepithema humile*, in North America more closely resemble those of *V. germanica* in Australia. As with *V. germanica*, *S. invicta* and *L. humile* were recently introduced and have expanded their ranges predominantly through accidental human transport (Lofgren 1986; Holway 1998). Investigations of the genetic structure of introduced *S. invicta* have uncovered strong and significant genetic structure for mitochondrial markers at sites separated by less than 1 km (Ross & Shoemaker 1997; Goodisman & Ross 1998; Ross *et al*. 1999). Moreover, moderate nuclear genetic differentiation also exists among populations separated by a few hundred km within the introduced range of the fire ant (Ross *et al*. 1999). Much of the mitochondrial structure is believed to result from the poor dispersal abilities of reproductive queens (Ross & Keller 1995a), and the significant nuclear genetic structure has been partly explained by the colonization history of *S. invicta* (Ross *et al*. 1999). In contrast to *S. invicta*, introduced *L. humile* apparently displayed little nuclear genetic structure over a scale of hundreds of kilometres (Tsutsui *et al*. 2000), although the relationship between genetic and geographical distance was not tested explicitly.

In addition to population genetic studies of social Hymenoptera, at least one genetic study of an introduced termite (Isoptera) has been undertaken. This investigation used allozymes to examine the genetic structure of the Formosan termite *Coptotermes formosanus* in the United States. The results revealed significant genetic differentiation among study populations (Korman & Pashley 1991), interpreted as evidence that *C. formosanus* had been introduced to the United States several times and, like *V. germanica*, had possibly experienced founder effects related to the introductions.

These studies, together with the present investigation, provide a glimpse of the population genetics of introduced social insects. Taken together, they suggest that genetic differentiation frequently accompanies invasion. Moreover, they indicate that bottlenecks occur not only during the initial introduction, but also during subsequent spreading. Rather than in a steady advancing wave, the colonization of a new habitat by invasive social insects appears to occur sporadically, wherein a few individuals initially become

established, then later suddenly and dramatically expand their range.

Acknowledgements

We thank P. Thorén for providing unpublished microsatellite primer sequences, A.D. Austin, R. Bashford, M.E. Carew, J.G. Ewen, J.K. Hatt, M. Kasper, M. Sanetra, P. Schmid-Hempel, R. Schmid-Hempel, P. Simmul, P.J. Spradbery and the Ararat Euro Wasp Research Group for providing samples, and L. Atkinson, J.G. Ewen, D.J. Futuyma, P. Taberlet, K.G. Ross, M. Sanetra and two anonymous reviewers for comments on earlier versions of this manuscript. This research was supported in part by a National Science Foundation Postdoctoral Fellowship in the Biosciences #DBI-9804263 (M.A.D.G.), a Commonwealth Scientific and Industrial Research Organization McMaster Fellowship (R.W.M.) and an Australian Research Council grant (R.H.C.).

References

Akre RD, MacDonald JF (1986) Biology, economic importance and control of yellow jackets. In: *Economic Impact and Control of Social Insects* (ed. Vinson SB), pp. 353–412. Praeger, New York.

Archer ME (1980) Population dynamics. In: *Social Wasps: Their Biology and Control* (ed. Edwards RE), pp. 172–207. Rentokil, East Grinstead, UK.

Beggs JR (2000) Impact and control of introduced *Vespula* wasps in New Zealand. In: *The Hymenoptera: Evolution, Biodiversity, and Biological Control* (eds Austin AD, Dowton M), pp. 404–409. CSIRO Publications, Melbourne.

Beggs JR, Wilson P (1991) The kaka (*Nestor meridionalis*), a New Zealand parrot endangered by introduced wasps and mammals. *Biological Conservation*, **56**, 23–38.

Chakraborty R, Nei M (1977) Bottleneck effects on average heterozygosity and genetic distance with the stepwise mutation model. *Evolution*, **31**, 347–356.

Clapperton BK, Moller H, Sandlant GR (1989) Distribution of social wasps (Hymenoptera: Vespidae) in New Zealand in 1987. *New Zealand Journal of Zoology*, **16**, 315–323.

Clarke GM, Grosse S, Matthews M *et al.* (2001) Environmental Pest Species in Australia. In: *Australia: State of the Environment, Second Technical Paper Series (Biodiversity)*. Department of the Environment and Heritage, Canberra.

Crosland MW (1991) The spread of the social wasp, *Vespula germanica*, in Australia. *New Zealand Journal of Zoology*, **18**, 375–388.

Crozier RH, Kaufmann B, Carew ME, Crozier YC (1999) Mutability of microsatellites developed for the ant *Camponotus consobrinus*. *Molecular Ecology*, **8**, 271–276.

Drake JA, Mooney HA, DiCastri F *et al.* eds (1989) *Biological Invasions: a Global Perspective*. John Wiley & Sons, Chichester, UK.

Edwards RE (1980) *Social Wasps: Their Biology and Control*. Rentokil, East Grinstead, UK.

Fordham RA (1991) Vespulid wasps at the upper forest margin in Tongariro National Park — a threat to the native biota? *New Zealand Journal of Zoology*, **18**, 151–153.

Goldstein DB, Linares AR, Cavalli-Sforza LL, Feldman MW (1995) An evaluation of genetic distances for use with microsatellite loci. *Genetics*, **139**, 463–471.

Goodisman MAD, Ross KG (1998) A test of queen recruitment models using nuclear and mitochondrial markers in the fire ant *Solenopsis invicta*. *Evolution*, **52**, 1416–1422.

Harris RJ (1991) Diet of wasps *Vespula vulgaris* and *V. germanica* in honeydew beech forest of the South Island, New Zealand. *New Zealand Journal of Zoology*, **18**, 159–170.

Harris RJ, Beggs JR (1995) Variation in the quality of *Vespula vulgaris* (L.) queens (Hymenoptera: Vespidae) and its significance in wasp population dynamics. *New Zealand Journal of Zoology*, **22**, 131–142.

Hartl DH, Clark AG (1989) *Principles of Population Genetics*. Sinauer Associates, Sunderland.

Holway DA (1998) Factors governing rate of invasion: a natural experiment using Argentine ants. *Oecologia*, **115**, 206–212.

Horwood MA, Toffolon RB, Brown GR (1993) Establishment and spread of *Vespula germanica* (F.) (Hymenoptera: Vespidae) in New South Wales and the influence of rainfall on its abundance. *Journal of the Australian Entomological Society*, **32**, 241–248.

Korman AK, Pashley DP (1991) Genetic comparisons among U.S. populations of Formosan subterranean termites. *Sociobiology*, **19**, 41–50.

Lewis PO, Zaykin D (2000) *Genetic Data Analysis: Computer program for the analysis of allelic data*, Version 1.0 (d15).

Lobo JA (1995) Morphometric, isozymic and mitochondrial variability of Africanized honeybees in Costa Rica. *Heredity*, **75**, 133–141.

Lobo JA, Del Lama MA, Mestriner MA (1989) Population differentiation and racial admixture in the Africanized honeybee (*Apis mellifera*). *Evolution*, **43**, 794–802.

Lofgren CS (1986) History of imported fire ants in the United States. In: *Fire Ants and Leaf-Cutting Ants: Biology and Management* (eds Lofgren CS, Vander Meer RK), pp. 36–47. Westview Press, Boulder, CO.

Matsuura M, Yamane S (1984) *Biology of the Vespine Wasps*. Springer-Verlag, New York, NY.

Michener CD (1974) *The Social Behavior of the Bees: a Comparative Study*. Belknap Press of Harvard University Press, Cambridge, MA.

Michod RE (1980) Evolution of interactions in family-structured populations: mixed mating models. *Genetics*, **96**, 27–296.

Moller H (1996) Lessons for invasion theory from social insects. *Biological Conservation*, **78**, 125–142.

Moller H, Tilley JAV (1989) Beech honeydew: Seasonal variation and use by wasps, honey bees, and other insects. *New Zealand Journal of Zoology*, **16**, 289–302.

Mooney HA, Drake JA, eds. (1986) *Ecology of Biological Invasions of North America and Hawaii*. Springer-Verlag, New York, NY.

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

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York, NY.

Oldroyd BP, Cornuet JM, Rowe D, Rinderer TE, Crozier RH (1995) Racial admixture of *Apis mellifera* in Tasmania, Australia: Similarities and differences with natural hybrid zones in Europe. *Heredity*, **74**, 315–325.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.

Ross KG, Carpenter JM (1991) Population genetic structure, relatedness, and breeding systems. In: *The Social Biology of Wasps* (eds Ross KG, Matthews RW), pp. 451–479. Comstock Publishing Associates, Ithaca, NY.

Ross KG, Keller L (1995a) Ecology and evolution of social organization: Insights from fire ants and other highly eusocial insects. *Annual Review of Ecology and Systematics*, **26**, 631–656.

Ross KG, Keller L (1995b) Joint influence of gene flow and selection on a reproductively important genetic polymorphism in the fire ant *Solenopsis invicta*. *American Naturalist*, **146**, 325–348.

Ross KG, Shoemaker DD (1997) Nuclear and mitochondrial genetic structure in two social forms of the fire ant *Solenopsis invicta*: insights into transitions to an alternate social organization. *Heredity*, **78**, 590–602.

Ross KG, Shoemaker DD, Krieger MJB, DeHeer CJ, Keller L (1999) Assessing genetic structure with multiple classes of molecular markers: a case study involving the introduced fire ant *Solenopsis invicta*. *Molecular Biology and Evolution*, **16**, 525–543.

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

Schiff NM, Sheppard WS, Loper GM, Shimanuki H (1994) Genetic diversity of feral honey bee (Hymenoptera: apidae) populations in the southern United States. *Annals of the Entomological Society of America*, **87**, 842–848.

Simon C, Frati F, Beckenbach A *et al*. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America*, **87**, 651–701.

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

Sokal RR, Rohlf FJ (1995) *Biometry*. W. H. Freeman and Co., New York, NY.

Spradbery JP (1973) *Wasps: an Account of the Biology and Natural History of Solitary and Social Wasps*. Sidgwick & Jackson, London.

Spradbery JP, Maywald GF (1992) The distribution of the European or German wasp, *Vespula germanica* (F.) (Hymenoptera: Vespidae), in Australia: Past, present and future. *Australian Journal of Zoology*, **40**, 495–510.

Thomas CR (1960) The European wasp (*Vespula germanica* Fab.) in New Zealand. *New Zealand Department of Scientific and Industrial Research Information Series*, **27**, 1–74.

Tsutsui ND, Suarez AV, Holway DA, Case TJ (2000) Reduced genetic variation and the success of an invasive species. *Proceedings of the National Academy of Sciences of the USA*, **97**, 5948–5953.

Vinson SB, ed. (1986) *Economic Impact and Control of Social Insects*. Praeger, New York, NY.

Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, **10**, 506–513.

Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**, 1358–1370.

Williams DF, ed. (1994) *Exotic Ants: Biology, Impact, and Control of Introduced Species*. Westview Press, Boulder, CO.

Williamson M (1996) *Biological Invasions*. Chapman & Hall, New York, NY.

This work comprises part of a larger study on introduced *Vespula* wasps in Australia. Michael Goodisman is a postdoctoral fellow at James Cook University examining social insect genetics, theoretical population biology and hybridization. Robert Matthews is Professor of Entomology at the University of Georgia and is interested in the behaviour, ecology and evolution of insects. Ross Crozier is Professor of Zoology at James Cook University and studies social insect biology, molecular evolution and conservation genetics.
