The Journal of Heredity

American Genetic Association

July/August 2001

Brief Communications

Reproduction and Recruitment in Perennial Colonies of the Introduced Wasp *Vespula germanica*

M. A. D. Goodisman, R. W. Matthews, J. P. Spradbery, M. E. Carew, and R. H. Crozier

We investigated the genetic structure of perennial colonies of the yellowjacket wasp (*Vespula germanica*) in its introduced range in Australia and New Zealand. The nuclear genotypes of 712 gynes from 21 colonies, 147 workers from 5 colonies, and 81 males from 4 colonies were assayed at three polymorphic microsatellite loci. The mitochondrial haplotypes of all wasps also were determined for a 450-bp region of the mtDNA using double-stranded conformational polymorphism (DSCP) analysis. We found that multiple reproductives were needed to explain the genotypes of gynes, workers, and males in 7 of 21, 2 of 5, and 2 of 4 colonies, respectively, and that nestmate relatedness of these three castes equaled 0.42, 0.16, and 0.22, respectively. The mitochondrial data revealed that all individuals shared the same mtDNA haplotype in 20 of the 21 colonies. However, in one colony, gynes and workers displayed multiple mtDNA haplotypes, indicating that nonnestmate recruitment had occurred. Overall the genetic structure within the majority of perennial colonies conformed to expectations based on the biology of *V. germanica* and kin selection theory for polygyne colonies; multiple reproductives successfully produced offspring and were recruited into their natal nests, thereby maintaining relatively high relatedness between interacting individuals.

Eusocial insect colonies are frequently headed by multiple reproductive queens (Crozier and Pamilo 1996). One explanation for the evolution of multiple-queen (polygyne) colonies is that environmental pressures place constraints on independent colony founding and lead to selection on queens to remain in their natal nests or enter already established nests (Herbers 1993; Pamilo 1991). These selective constraints probably vary between habitats. Thus eusocial insects in novel environments may experience new selection pressures, which may lead to variation in colony queen number.

The eusocial wasp *Vespula germanica* (commonly known as the European wasp or the German yellowjacket) shows differences in colony queen number, which are apparently related to environmental conditions (Spradbery 1973). In native populations found in the cooler regions of Europe and Asia, *V. germanica* colonies are headed by a single queen and display an annual life cycle (Greene 1991). However, in the warmer climates of the wasp's native and introduced ranges, colonies may persist for more than a single season (Harris 1996; Plunkett et al. 1989; Spradbery 1991). These perennial, overwintering colonies exhibit many important differences from the annual colonies from which they are derived (reviewed in Greene 1991; Spradbery 1991). For example, they grow much larger than annual colonies; whereas annual colonies may contain a few thousand workers, perennial colonies may contain well over 100,000 workers. Perennial colonies also continue to produce sexual offspring throughout the winter and then revert to worker production in the spring. Finally, of particular significance to this study, perennial colonies may be headed by multiple queens, with hundreds of such reproductives present in some cases.

The purpose of this study was to investigate the effects of polygyny on the genetic structure of perennial *V. germanica* colonies. Specifically we assayed the nuclear and mitochondrial genotypes of wasps to ascertain if multiple queens or workers successfully contributed to offspring production and to determine the genetic relationships of nestmates. We expect that multiple reproductives will be active within colonies and that nestmates will be closely related, thereby maintaining conditions that allow workers to obtain relatively high inclusive fitness benefits.

Methods

Mature reproductive queens and prereproductive females (jointly denoted as gynes), workers, and males were collected from overwintering nests from 12 populations in the introduced range of *V. germanica* in Australia and New Zealand (Table 1). In total, 33.90 $\pm$ 12.04 ($\bar{x} \pm$ SD) gynes were sampled from 21 nests, 31.40 $\pm$ 12.64 workers were sampled from five nests, and 20.25 $\pm$ 13.82 males were sampled from four nests. The nuclear genotype of each wasp was assayed at three microsatellite loci: *Rufa 5, Rufa 18,* and *Rufa 19* (Goodisman et al. 2001). Allele frequencies within populations were calculated using the program Relatedness 4.2 (Queller and Goodnight 1989), and the expected heterozygosity, $H = 1 - \Sigma_i p_i^2$, where p_i is the average frequency of allele i across all 12 populations, was used as a measure of the variability for each of the nuclear markers.

The nuclear genotypic data were analyzed to determine if more than a single queen (mated egglayer) contributed to the production of gynes or workers within nests, and if multiple gynes or workers produced males (*Vespula* gynes and workers, which are diploid, may only be produced by queens, but males, which are haploid, may be produced by both gynes and workers; Spradbery 1991). Multiple queens were deemed responsible for gyne or worker production within colonies if the sample of gynes or workers displayed at least three distinct genotypes that

Table 1. Approximate locations of 21 perennial *V. germanica* colonies and number of gynes (G), workers (W), and males (M) sampled from each colony

Population location	Colony ID	Caste	Sample size
33°25′S, 149°35′E (AUS)	1	G	40
	2	G	40
	3	G	30
	4	G	40
34°27′S, 150°27′E (AUS)	5	G	40
35°51′S, 151°15′E (AUS)	6	G	40
34°27′S, 150°56′E (AUS)	7	G	40
37°05′S, 144°13′E (AUS)	8	G	40
	9	G	40
		W	25
	10	G	40
	11	G	40
		M	15
	12	G	40
	13	G	40
37°39′S, 145°31′E (AUS)	14	G	40
		M	40
		W	40
— (AUS)	15	G	40
		W	12
— (AUS)	16	G	40
43°32′S, 172°38′E (NZ)	17	G	2
		M	8
	18	G	40
		M	18
		W	40
43°39′S, 172°29′E (NZ)	19	G	13
		W	40
43°18′S, 172°11′E (NZ)	20	G	14
46°24′S, 168°24′E (NZ)	21	G	13

AUS = Australia, NZ = New Zealand, — = exact location unknown.

shared no alleles at any locus. Multiple gynes or workers were judged as contributing to male production within colonies if the sample of males displayed at least three different alleles at any locus. The relatedness of nestmate gynes, workers, and males was estimated using RELATEDNESS 4.2 (Queller and Goodnight 1989). Differences in allele frequencies across populations were taken into account by using the "deme" function. Groups (colonies) were weighted equally and standard errors for estimates were obtained by jackknifing over colonies.

Double-stranded conformation polymorphism (DSCP) analysis was used to determine each wasp's haplotype at a PCR-amplified mitochondrial DNA (mtDNA) fragment (Atkinson and Adams 1997). Genomic DNA for PCR amplification was extracted from single *V. germanica* legs using a variation of the Chelex protocol as described by Crozier et al. (1999). An approximately 450 bp fragment of mtDNA, which spanned the intergenic spacer region between cytochrome *b* and ND1, was amplified using the degenerate primers CB3Ext and tRs2 (Chiotis et al. 2000). To visualize the fragment, the primer CB3Ext was first end-labeled with [γ^{33}P]-ATP. PCRs were then carried out in a final volume of 10 μl containing 2 μl of genomic DNA, 0.4 U *Taq* DNA polymerase, and a final concentration of 167 μM dNTPs, 0.8 μM of unlabeled tRs2, 0.2 μM of unlabeled CB3Ext, 0.06 μM of radioactively end-labeled CB3Ext, and 1× Promega buffer (with 1.5 mM $MgCl_2$). The PCR cycling profile began with an initial denaturation at 94°C for 2 min and then proceeded with 35 cycles of 93°C for 30 sec, 35°C for 30 sec, and 72°C for 1 min, followed by a final extension of 72°C for 10 min. PCR products were electrophoresed on a 5% glycerol, 1× TBE, 9% nondenaturing polyacrylamide (39:1 acrylamide:*bis*-acrylamide) gel at a constant power of 10 W for about 24 h at room temperature. The individual mitochondrial haplotypes were scored after exposing the dried gel to Kodak film overnight.

The mitochondrial data were then examined to determine if nestmates shared the same haplotype, a result that would be consistent with all nestmates being descended from the single, original, founding queen of the colony. In contrast, the presence of at least two mitochondrial haplotypes signaled that wasps were part of at least two extended maternal lineages. In cases where multiple haplotypes were detected, an allelic probability test as implemented by the program GENEPOP 3.2 (Raymond and Rousset 1995) was used to determine if wasps possessing distinct haplotypes differed genetically at their microsatellite loci. We also used a *G*-test of independence to ascertain if the mtDNA haplotype frequencies differed among castes.

Results and Discussion

Reproductive Patterns

The three nuclear loci *Rufa 5, Rufa 18,* and *Rufa 19* exhibited 6, 9, and 9 alleles, respectively (Table 2), and their associated expected heterozygosities (*H*) equaled 0.56, 0.66, and 0.74. Thus the markers displayed sufficient variation for studying the reproductive processes occurring within polygyne *V. germanica* colonies.

We first examined the nuclear genotypic data directly to determine if multiple reproductives were active within colonies. Our analyses indicated that at least two queens were necessary to explain the genotypes of gynes in 7 of the 21 colonies and of workers in 2 of the 5 colonies. Moreover, multiple gynes or workers were responsible for the production of males in two of the four colonies from which males were obtained (Table 2). These proportions probably represent an underestimate of the fraction of nests containing multiple reproductives, because the genetic markers we used displayed limited variation and the number of wasps sampled per nest was finite. Moreover, our ability to detect multiple reproductives was further diminished because nestmates were related. These problems also prevented the accurate estimation of the actual number of reproductives that were active within colonies.

Previous studies in *Vespula* found that many gynes within perennial nests were inseminated or possessed well-developed ovaries (Ross and Matthews 1982; Ross and Visscher 1983; Ratnieks and Miller 1993; Ratnieks et al. 1996; Vetter and Visscher 1997). In addition, the large number of wasps within some perennial *Vespula* nests strongly suggested the presence of multiple reproductives (Greene 1991; Spradbery 1991). Thus the genetic confirmation that multiple queens produced offspring within these nests was not unexpected.

Genetic Relationships of Nestmates

The relatedness (±SE) of nestmate gynes, 0.42 ± 0.095, differed significantly from 0.0 (t_{12} = 4.65, $P < .001$) and 0.75 (t_{12} = 3.22, $P = .0072$), the values expected if all gynes were unrelated or full sisters, respectively. *V. germanica* queens normally mate with multiple males (Goodisman MAD, et al., unpublished data). Thus the relatedness estimate indicated that nestmate gynes were close relatives. This result conforms to previous findings in other polygyne wasps, where nestmate queens were usually significantly related (reviewed by Crozier and Pamilo 1996). The estimates of worker relatedness (0.16 ± 0.12) and male relatedness (0.22 ± 0.10) were both based on relatively few colonies. Consequently we did not possess sufficient power to determine if the worker and male values differed significantly from zero. Nevertheless, the relatively high relatedness of gynes suggests that polygyny does not necessarily lead to a rapid decline in nestmate relatedness in perennial *V. germanica* colonies.

Analysis of the mitochondrial DSCP uncovered two distinguishable haplotypes, hereafter denoted as fast (*F*) and slow (*S*), within the population (Table 2). The estimated frequencies of *F* and *S* across all populations combined equaled 0.45 and 0.55, respectively. We studied the distribution of mitochondrial haplotypes within nests to detect the presence of multiple,

Table 2. Allele frequencies at three microsatellite markers (*Rufa 5*, *Rufa 18*, and *Rufa 19*) and haplotype frequencies at a single mitochondrial marker (mtDNA) for gynes (G), workers (W), and males (M) sampled from 21 perennial *V. germanica* colonies

Colony and caste	*Rufa 5*						*Rufa 18*									*Rufa 19*									mtDNA	
	143	145	147	149	177	195	187	193	195	197	199	201	203	205	211	194	198	200	202	204	206	208	210	212	*F*	*S*
1 G		0.22		0.78							0.20		0.80					0.71			0.28			0.01	1.0	
2 G		0.71		0.29							0.46	0.03	0.51						0.77		0.23					1.0
3 G		0.05		0.57	0.38								1.0					0.62	0.27		0.11				1.0	
4 G		0.11		0.89							0.11		0.88	0.01				0.23	0.28		0.11			0.38	1.0	
5 G		0.86	0.01			0.13	0.01	0.01		0.03	0.71	0.24						0.27	0.04			0.49	0.20			1.0
6 G		0.60		0.40							0.41	0.25	0.34					0.05	0.88	0.06	0.01					1.0
7 G		0.15	0.66	0.19							0.67	0.33						0.31	0.49		0.01	0.19			1.0	
8 G*		0.97		0.03			0.18			0.19	0.26	0.37							0.70	0.29	0.01				1.0	
9 G*†		0.70	0.23	0.06	0.01		0.04		0.01	0.06	0.80	0.09						0.01	0.46	0.10	0.13			0.30	0.45	0.55
9 W*†		0.70	0.22	0.08			0.05			0.15	0.72	0.04	0.04					0.02	0.44	0.20	0.1			0.24	0.28	0.72
10 G*		0.69	0.01	0.30						0.01	0.59	0.26	0.14					0.27	0.45		0.05			0.23		1.0
11 G*		0.78	0.04	0.10	0.08		0.10			0.38	0.32	0.11	0.09			0.01		0.04	0.53	0.10	0.03			0.29	1.0	
11 M*		0.85			0.15		0.50			0.50									0.38	0.23				0.39	1.0	
12 G	0.06	0.71				0.23					0.23	0.51	0.26					0.33				0.26	0.41		1.0	
13 G		1.0								0.74	0.26									0.74			0.26		1.0	
14 G*		0.46	0.04	0.50						0.20	0.36	0.09	0.35					0.04	0.7		0.14			0.12		1.0
14 M*		0.45	0.03	0.53						0.20	0.48	0.17	0.15					0.03	0.87	0.05				0.05		1.0
14 W*	0.01	0.53	0.04	0.42			0.06		0.04	0.09	0.41	0.21	0.19					0.03	0.79	0.08	0.02			0.08		1.0
15 G*		0.8	0.01	0.08		0.11	0.05	0.06		0.03	0.67	0.11	0.04		0.04			0.51	0.05		0.01	0.29	0.14		1.0	
15 W		0.79		0.08		0.13	0.13	0.13		0.04	0.41	0.25	0.04					0.58	0.04			0.21	0.17		1.0	
16 G*		0.58	0.07	0.11	0.24				0.02	0.28	0.26	0.27	0.17			0.03		0.22	0.12	0.11	0.50			0.02	1.0	
17 G	0.25	0.75									1.0								0.75					0.25		1.0
17 M	0.25	0.75									1.0								0.37					0.63		1.0
18 G	0.05	0.92	0.03								0.74		0.26				0.06	0.45	0.48					0.01		1.0
18 M	0.11	0.89									0.47		0.53					0.72	0.28							1.0
18 W	0.05	0.95									0.73	0.01	0.26				0.01	0.5	0.4					0.09		1.0
19 G	0.11	0.77	0.12								0.69		0.31					0.11	0.89							1.0
19 W	0.13	0.87									0.79		0.21					0.14	0.86							1.0
20 G	0.25	0.75									1.0							0.43	0.57							1.0
21 G	0.65	0.35									0.77		0.23					0.77	0.23							1.0

Allele names for microsatellite markers correspond to the size of the PCR-amplified product.

* Indicates that nestmates could not have been produced by a single reproductive.

† Indicates that nestmates could not have been part of a single extended matriline (see text for details).

extended, maternal lineages. We discovered only a single colony where wasps segregated for both the *F* and *S* haplotypes (colony 9; Table 2). As was the case with our test for multiple reproductives (see above), the modest level of variation at the mitochondrial locus and finite sample sizes taken from each colony resulted in limited power to detect multiple extended maternal lineages. Consequently some colonies possessing such lineages probably remained undetected.

The allelic probability test confirmed that both gynes and workers from colony 9 that possessed distinct haplotypes differed genetically at their nuclear loci ($P < .001$ for both castes), a result consistent with the presence of multiple extended maternal lineages within the colony. Examination of the genotypes of gynes and workers within each matriline revealed that the nuclear genotypes of wasps with the *F* haplotype could parsimoniously be explained by the presence of a single queen. However, at least two queens were necessary to account for the genotypes of wasps possessing the *S* haplotype. The frequency of the *F* haplotype among gynes and workers in colony 9 was 0.45 and 0.28, respectively. These frequencies did not differ significantly ($G_1 = 1.92$, $P = .17$), which suggested an absence of reproductive skew among the queens within this colony.

At least three mechanisms could lead to both gynes and workers within colonies possessing multiple mitochondrial haplotypes. First, two queens with different mitochondrial haplotypes could have cofounded the perennial colony, and these queens could have then coexisted in a polygyne association. This explanation, however, is inconsistent with the known biology of *V. germanica.* Annual colonies, from which perennial colonies are necessarily derived, are initiated and headed by only a single queen (Greene 1991).

The second explanation for the presence of multiple mitochondrial haplotypes within a single colony is that many unrelated gynes and workers joined an already established colony. Previous genetic studies in *V. germanica* failed to detect the acceptance of nonnestmate workers in annual colonies (Goodisman MAD, et al., unpublished data). However, evidence from the honeybee (*Apis mellifera*) supports the hypothesis that workers and males may be accepted into foreign colonies (reviewed by Moritz and Neumann 1996). Thus, although this explanation seems unlikely in *V. germanica,* it cannot be rejected conclusively.

Finally, one or few nonnestmate queens (as opposed to many gynes and workers) may have entered the nest and subsequently produced new gynes and workers. Evidence for this behavior in polygyne *Vespula* already exists. In two cases, several queens were discovered in colonies that apparently had not started producing sexuals. Consequently it was suggested that these queens originated from foreign colonies (Ross and Matthews 1982; Spradbery 1991). The patterns of queen recruitment in *Vespula* thus parallel those observed in some ants, in which colonies occasionally present more than one mitochondrial type, indicating the infrequent immigration of unrelated females (e.g., Carew et al. 1997; Stille and Stille 1992; Tay et al. 1997).

Conclusion

This study examined the genetic structure of perennial colonies of the introduced wasp *V. germanica.* The genetic data indicated that multiple functional queens frequently headed these colonies. Also, nest-

mate gynes were closely related and all wasps usually belonged to a single extended matriline presumably originating from the original founding queen. The overall structure of perennial *V. germanica* colonies was broadly consistent with expectations based on kin selection theory within polygyne colonies. Multiple queens successfully reproduced, and new queens were usually recruited into their natal nests so that workers raised relatives and received high inclusive fitness benefits.

From the Department of Genetics, La Trobe University, Bundoora, Victoria, Australia (Goodisman, Carew, and Crozier), Department of Zoology and Tropical Ecology, James Cook University, Townsville, Queensland, Australia (Goodisman and Crozier), Department of Entomology, University of Georgia, Athens, Georgia (Matthews), and Division of Entomology, Commonwealth Scientific and Industrial Research Organization, Canberra, Australian Capital Territory, Australia (Spradbery). We thank P. Thorèn for providing unpublished microsatellite primer sequences, J. Ewen, J. Hatt, and R. Johnson for field assistance, S. Harcourt for samples from New Zealand, and L. Atkinson, J. Ewen, J. Hatt, M. Sanetra, and G. Thompson for helpful discussion and comments on earlier versions of this manuscript. This research was supported in part by a National Science Foundation Postdoctoral Fellowship in the Biosciences no. DBI-9804263 (to M.A.D.G.) and a Commonwealth Scientific and Industrial Research Organization McMaster Fellowship (to R.W.M.). Address correspondence to Michael A. D. Goodisman, Department of Biochemistry, 440 Biological Sciences West, P.O. Box 210088, University of Arizona, Tucson, AZ 85721-0088, or e-mail: goodisma@email.arizona.edu.

References

Atkinson L and Adams E, 1997. Double-strand conformation polymorphism (DSCP) analysis of the mitochondrial control region generates highly variable markers for population studies in a social insect. Insect Mol Biol 6:369–376.

Carew ME, Tay WT, and Crozier RH, 1997. Polygyny via unrelated queens indicated by mitochondrial DNA variation in the Australian meat ant *Iridomyrmex purpureus.* Insect Soc 44:7–14.

Chiotis M, Jermiin LS, and Crozier RH, 2000. A molecular framework for the phylogeny of the ant subfamily Dolichoderinae. Mol Phyl Evol 17:108–116.

Crozier RH, Kaufmann B, Carew ME, and Crozier YC, 1999. Mutability of microsatellites developed for the ant *Camponotus consobrinus.* Mol Ecol 8:271–276.

Crozier RH and Pamilo P, 1996. Evolution of social insect colonies: sex allocation and kin selection. Oxford: Oxford University Press.

Goodisman MAD, Matthews RW, and Crozier RH, 2001. Hierarchical genetic structure of the introduced wasp *Vespula germanica* in Australia. Mol Ecol. 10:1423–1432.

Greene A, 1991. *Dolichovespula* and *Vespula.* In: The social biology of wasps (Ross KG and Matthews RW, eds). Ithaca, NY: Comstock; 263–305.

Harris RJ, 1996. Frequency of overwintered *Vespula germanica* (Hymenoptera: Vespidae) colonies in scrubland-pasture habitat and their impact on prey. N Z J Zool 23:11–17.

Herbers JM, 1993. Ecological determinants of queen number in ants. In: Queen number and sociality in insects (Keller L, ed). Oxford: Oxford University Press; 262–293.

Moritz RFA and Neumann P, 1996. Genetic analysis of the drifting of drones in *Apis mellifera* using multilocus DNA fingerprinting. Ethology 102:580–590.

Pamilo P, 1991. Evolution of colony characteristics in social insects. II. Number of reproductive individuals. Am Nat 138:412–433.

Plunkett GM, Moller H, Hamilton C, Clapperton BK, and Thomas CD, 1989. Overwintering colonies of German (*Vespula germanica*) and common wasps (*Vespula vulgaris*) (Hymenoptera: Vespidae) in New Zealand. N Z J Zool 16:345–353.

Queller DC and Goodnight KF, 1989. Estimating relatedness using genetic markers. Evolution 43:258–275.

Ratnieks FLW and Miller DG, 1993. Two polygyne overwintered nests of *Vespula vulgaris* from California. Psyche 100:43–50.

Ratnieks FLW, Vetter RS, and Visscher PK, 1996. A polygynous nest of *Vespula pensylvanica* from California with a discussion of possible factors influencing the evolution of polygyny in *Vespula.* Insect Soc 43:401–410.

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

Ross KG and Matthews RW, 1982. Two polygynous overwintered *Vespula squamosa* colonies from the southeastern U.S. (Hymenoptera: Vespidae). Florida Entomol 65:176–184.

Ross KG and Visscher PK, 1983. Reproductive plasticity in yellowjacket wasps: a polygynous, perennial colony of *Vespula maculifrons.* Psyche 90:179–191.

Spradbery JP, 1973. Wasps: an account of the biology and natural history of solitary and social wasps. London: Sidgwick & Jackson.

Spradbery JP, 1991. Evolution of queen number and queen control. In: The social biology of wasps (Ross KG and Matthews RW, eds). Ithaca, NY: Comstock; 336–388.

Stille M and Stille B, 1992. Intra- and inter-nest variation in mitochondrial DNA in the polygynous ant *Leptothorax acervorum* (Hymenoptera; Formicidae). Insect Soc 39:335–340.

Tay WT, Cook JM, Rowe DJ, and Crozier RH, 1997. Migration between nests in the Australian arid-zone ant *Rhytidoponera* sp. 12 revealed by DGGE analyses of mitochondrial DNA. Mol Ecol 6:403–411.

Vetter RS and Visscher PK, 1997. Plasticity of annual cycle in *Vespula pensylvanica* shown by a third year polygynous nest and overwintering of queens inside nests. Insect Soc 44:353–364.

Received October 2, 2000
Accepted April 30, 2001

Corresponding Editor: Stephen Schaeffer

Recombination Between Two Amplified Esterase Alleles in *Culex pipiens*

C. Berticat, M. Marquine, M. Raymond, and C. Chevillon

Esterase gene amplification at the *Ester* superlocus provides organophosphate resistance in the mosquito *Culex pipiens* (L.). In this study we explored the possibility of recombination between two amplified esterase alleles, thus generating a composite amplified allele. To do that, females heterozygous for two distinct amplified alleles (*Ester*^{2} and *Ester*^{4}) were crossed with males homozygous for a third resistance allele (*Ester*^{8}). Among analyzed offspring, one recombinant composite allele (*Ester*$^{2–4}$) was detected, providing a rate of recombination of approximately 0.2%. This is the first report of a recombination between two distinct amplified esterase alleles. This phenomenon renders the predictability of allele evolution considerably more complex than was previously thought.

General models of population genetics that try to infer the outcome of adaptive genes are often based on simple approximations, like the existence of one or two loci with two alleles. Concerning resistance to organophosphorous (OP) insecticides in *Culex pipiens* mosquitoes, the adaptive system is more complex. The *Ester* superlocus, which is one of the main genome areas involved in this resistance (Lenormand et al. 1998), presents multiple resistance alleles. This superlocus is in fact composed of two loci on chromosome II, *Est-3* and *Est-2,* separated by an intergenic DNA fragment of 2–6 kb (Guillemaud et al. 1997; Heyse et al. 1996; Rooker et al. 1996) and both loci encode for detoxifying esterases A and B, respectively. The resistance conferred by *Ester* is due to an esterase overproduction that is the result of two nonexclusive mechanisms: gene amplification of one (*Est-2*) or both loci, or change in gene regulation (for a review, see Raymond et al. 1998). Six *Ester* alleles involved in resistance have been described: four correspond to the coamplification of both *Est-2* and *Est-3* loci (*Ester*^{2}, *Ester*^{4}, *Ester*^{5}, and *Ester*^{8}, encoding esterases A2-B2, A4-B4, A5-B5, and A8-B8, respectively), one corresponds to the exclusive amplification of *Est-2* (*Ester*^{B1}, encoding esterase B1), and one corresponds to an upregulation of *Est*-3 (*Ester*^{1}, encoding esterase A1). Two other amplified esterase genes have been reported, but direct comparison with the above alleles is needed to confirm that they are distinct alleles (Vaughan et al. 1997; Xu et al. 1994). *Est-3* and *Est-2* loci have always been found in maximal linkage disequilibrium for alleles involved in resistance in field studies (see review in Chevillon et al. 1999; Raymond et al. 1998), which justifies the concept of *Ester* superlocus.

Recently molecular data have confirmed that the amplification level of amplified alleles is variable in natural populations (Callaghan et al. 1998; Weill et al. 2000). Moreover, level of resistance, esterase ac-