present in the five specimens analyzed and absent in six *D. persimilis* and seven *D. pseudoobscura* of different origin. The number of specimens analyzed is small for *D. miranda,* but since the lines used came from different localities (see Materials and Methods), it can be assumed that these bands are diagnostic. We estimated the size of species-specific bands observed by Pascual et al. (1997b) in *D. athabasca* and *D. azteca.* A total of nine bands clearly identified *D. athabasca* and seven bands identified *D. azteca* (Table 1).

The amount of variation between *D. pseudoobscura* and *D. persimilis* was estimated using the analysis of molecular variance. *D. miranda* was not included in the analysis due to the low number of individuals. Only polymorphic bands were used in the analysis. Of the overall phenotypic variation, 58.38% was due to variation between species and 41.62% to intraspecific variation. Population structure was not analyzed because the number of individuals was very low in some localities. Significant genetic differentiation between species was detected ($\phi_{ST} = 0.584$, $p < .001$). Tests of significance for variance components are based on 1,000 random permutations.

A total of 39 males and females from Bellingham (WA) and Salem (OR) kept in 70% ethanol were amplified with some of the diagnostic primers. The aim of this analysis was to increase the number of *D. miranda* in the sample. Males were morphologically classified to the subgroup level ahead of time and then the result of the RAPD amplification was cross-checked with the previous identification in order to assess the reliability of the system. A blind experiment, previously carried out with *D. azteca* and *D. athabasca* males using a morphological character and comparing the results with RAPD banding pattern has also shown the value of the technique (Pascual et al. 1997b). Bellingham (WA) individuals were classified as follows: 4 *affinis* subgroup males as *D. athabasca,* 16 *pseudoobscura* subgroup males as *D. pseudoobscura,* and 8 *obscura* group females as 7 *D. athabasca* and 1 *D. pseudoobscura.* Salem (OR) males, from the *pseudoobscura* subgroup, were classified as 7 *D. pseudoobscura* and 4 *D. persimilis.* All individuals were amplified with at least two primers, those producing species-specific bands. In each case opa-4 yielded a scorable set of bands for *D. athabasca,* opa-7 for *D. pseudoobscura,* opa-9 and opa-16 for *D. persimilis* and opb-8 for *D. miranda* (Table 1, Figure 1). Unfortunately no *D. miranda* was found and thus we could not increase the sample size of that species.

Here we describe species-specific bands of *D. pseudoobscura, D. persimilis,* and *D. miranda* that discriminate between these three sibling species. These results, along with those of a previous study using *D. azteca* and *D. athabasca* (Pascual et al. 1997b) permit classification of all the specimens collected in all samples of the distribution range of these five Nearctic species of the *obscura* group. We may thus be able to study their population dynamics and assess its influence on the colonizing success of *D. subobscura.*

From the Departament de Genètica, Universitat de Barcelona, Av. Diagonal 645, 08028 Barcelona, Spain. Address correspondence to Marta Pascual at the address above or e-mail: mpascual@porthos.bio.ub.es. This work was supported by grant PB96-0793-C04-03 from the DGES, Spain. We thank E. Haring, R. Huey, M. Noor, C. Segarra, and D. Sperlich for providing us with flies of different origin. We thank R. J. MacIntyre for his helpful comments and suggestions.

References

Anderson WW, Ayala FJ, and Michod RE, 1977. Chromosomal and allozymic diagnosis of three species of *Drosophila: D. pseudoobscura, D. persimilis,* and *D. miranda.* J Hered 68:71–74.

Armstrong J, Gibbs A, Peakall R, and Weiller G, 1996. RAPDistance programs: version 1.04 for the analysis of patterns of RAPD fragments. Canberra, Australia: Australian National University.

Barrio E, Latorre A, Moya A, and Ayala FJ, 1992. Phylogenetic reconstruction of the *Drosophila obscura* group, on the basis of mitochondrial DNA. Mol Biol Evol 9:621–635.

Beckenbach AT and Prevosti A, 1986. Colonization of North America by the European species *D. subobscura* and *D. ambigua.* Am Midl Nat 115:10–18.

Beckenbach AT, Wei YW, and Liu H, 1993. Relationships in the *Drosophila obscura* species group, inferred from mitochondrial cytochrome oxidase II sequences. Mol Biol Evol 10:619–634.

Buzzati-Traverso AA and Scossiroli R, 1955. The "obscura group" of the genus *Drosophila.* Adv Genet 7:47–92.

Dobzhansky TH and Epling C, 1944. Contributions to the genetics, taxonomy and ecology of *Drosophila pseudoobscura* and its relatives. Publications of the Carnegie Institute of Washington 554:1–183.

Excoffier L, 1995. WINAMOVA version 1.55. Genetics and biometry. Geneva, Switzerland: University of Geneva.

Latorre A, Barrio E, Moya A, and Ayala FJ, 1988. Mitochondrial DNA evolution in the *Drosophila obscura* group. Mol Biol Evol 5:717–728.

Palacios C and González-Candelas F, 1997. Analysis of population genetic structure and variability using RAPD markers in the endemic and endangered *Limonium dufourii* (Plumbaginaceae). Mol Ecol 6:1107–1121.

Pascual M, Ayala FJ, Prevosti A, and Serra L, 1993. Colonization of North America by *D. subobscura*: ecological analysis of three communities of drosophilids in California. J Zool Syst Evol Res 32:44–50.

Pascual M, Balanyà J, Latorre A, and Serra L, 1997a. Analysis of the variability of *Drosophila azteca* and *Drosophila athabasca* populations revealed by random amplified polymorphic DNA. J Zool Syst Evol Res 35:159–164.

Pascual M, Balanyà J, Latorre A, and Serra L, 1997b. Diagnosis of sibling species of *Drosophila* involved in the colonization of North America by *Drosophila subobscura.* Mol Ecol 6:293–296.

Powell JR, 1983. Interspecific cytoplasmic gene flow in the absence of nuclear gene flow: evidence from *Drosophila.* Proc Natl Acad Sci USA 80:492–495.

Prakash S, 1977. Genetic divergence in closely related sibling species *Drosophila pseudoobscura, D. persimilis* and *D. miranda.* Evolution 31:14–23.

Prevosti A, Serra L, Aguadé M, Ribó G, Mestres F, Balañà J, and Monclús MG, 1989. Colonization and establishment of the palearctic species *D. subobscura* in North and South America. In: Evolutionary biology of transient unstable populations (Fontdevila A, ed). Berlin: Springer-Verlag; 114–129.

Wang RL and Hey J, 1996. The speciation history of *Drosophila pseudoobscura* and close relatives: inferences from DNA sequence variation at the period locus. Genetics 144:1113–1126.

Received February 18, 1999
Accepted May 31, 1999

Corresponding Editor: Ross MacIntyre

Reproductive and Mate Choice Strategies in the Hermaphroditic Flatworm *Echinostoma caproni*

S. Trouvé, F. Renaud, P. Durand, and J. Jourdane

Due to the important role that mating systems play in the evolution of species, we investigate the selfing rate and mate choice in the simultaneous hermaphroditic parasite *Echinostoma caproni* (Trematoda). The echinostomes were maintained in two situations in mice: (1) double infections where the two individuals do or do not belong to the same geographic area isolate, and (2) triple infections where two of the three individuals originate from the same isolate and the third one originates from a different isolate. This experimental design permits analysis of intra- and interisolate selfing rates and of mate preference. We predict, in the first experiment, no difference between intra- and interisolate selfing rates. In the second experiment we expect a preferential outcrossing between individuals originating from the same isolate in order to avoid hybrid breakdown. The results obtained corroborate our predictions and emphasize the important and synergistic roles of selfing, inbreeding depression, and hybrid breakdown in the evolution of echinostome reproductive strategies.

Reproductive strategies constitute a major factor shaping the evolution of organisms. Indeed, mating systems influence the genetic variability as well as the genetic structure of populations (Charlesworth and Charlesworth 1987; Jarne 1995). In this context, hermaphrodites offer the opportunity to investigate the evolution of sexual reproductive modes since a single individual can self- or cross-fertilize. Compared to plants, few studies have been conducted on mating systems in animals. These works have been mostly carried out on molluscs [see Jarne et al. (1993) for a review; Doums et al. 1996; Städler et al. 1995), ascidians (Bishop 1996; Bishop et al. 1996), or free-living flatworms (Michiels and Streng 1998), and the analysis of reproductive strategies in helminth parasites has been roughly ignored for a long time. Nevertheless, studies on reproduction in parasites will provide data to fill out works on animals and to investigate if theories usually advanced to understand the evolution of mating systems in free-living organisms have a parasitic counterpart.

Some experiments have been conducted to investigate the mating behavior of the hermaphroditic platyhelminth parasite *Echinostoma caproni* (Trouvé et al. 1996). Infections of mice with two individuals, from two isolates (i.e., originating from two different geographic areas) showed that, in this condition, the echinostomes exhibit an unrestrictive mating system involving both selfing and outcrossing (Trouvé et al. 1996). Furthermore, in a mate-choice experiment where mice were infected with three individuals, two of the same isolate and one of another, we found that both related individuals produced very few outcrossed offspring with the unrelated individual (Trouvé et al. 1996). Although it was impossible to distinguish selfed from outcrossed offspring between related individuals, this result likely suggests a marked mate preference between individuals of the same isolate. This prezygotic isolation seems to be followed by a postzygotic isolation characterized by a hybrid breakdown. Indeed, it has been shown that the hybrids of the second and third generations display a significantly lower fecundity compared to both parental isolates and to the F_1 (i.e., hybrid breakdown; Trouvé et al. 1998).

In this context we can describe the forces which should drive the evolution of the echinostomes' reproductive strategies as follows. First, self-fertilization ensures the reproduction of the echinostomes, which, like many parasites, often evolve in low-density populations (Charnov et al. 1976; Ghiselin 1974; Tomlinson 1966). Second, inbreeding depression decreases the fitness of self-fertilized offspring. Third, after a period of isolated evolution, hybrid breakdown should reduce genetic exchange between different isolates.

Consequently, in the light of these predictions and our knowledge of echinostomes, we present a study on individuals originating from a new natural population, discovered in May 1996 in Mali, showing an isoenzymatic polymorphism. We investigate selfing versus outcrossing rate in (1) double infections where the two individuals do or do not belong to the same isolate; and (2) triple infections where two of the three individuals originate from the same isolate.

If our predictions are correct, we expect, in the first experiment, no difference between intra- and interisolate selfing rate. In the second experiment, we expect a preferential outcrossing between individuals originating from the same isolate in order to avoid hybrid breakdown.

Discussion

Echinostomes are simultaneous hermaphroditic Trematoda (Platyhelminth) parasitizing vertebrate intestines. The life cycle of *E. caproni* includes three successive hosts and an asexual reproduction occurring in the first intermediate host. *E. caproni* is routinely cycled in our laboratory according to standard procedures (Trouvé et al. 1996) using *Biomphalaria arabica* snails which act as first and second intermediate hosts and mice (Swiss OF1 stock) as the final host. In this study four isolates of *E. caproni* were used; they originate from Mali (Em), Egypt (El), Madagascar (Ec), and Cameroon (Ek) (Trouvé and Coustau 1998). The isolates El, Ec, and Ek are homozygous at the loci *GPI, PGM, MPI,* PheLeu peptidase, and PhePro peptidase and present at these loci, respectively, the alleles: El (aabaa), Ec (baaaa), and Ek (abcbb). Furthermore, the Em isolate has two homozygous electrophoretic forms (Em1 and Em2) for which the alleles at the previously mentioned loci are Em1 (aabaa) and Em2 (abbaa). The Em isolate also presents heterozygous individuals at the *PGM* locus.

Selfing Rate

We carried out infections of mice with two Em individuals (i.e., Em1 and Em2) to estimate the selfing rate involved within an isolate. Twenty days postinfection the intestines of the mice were opened and the worms collected. The uterus of each adult was torn to collect the eggs. Thirty larvae hatched from the eggs of each worm were individually brought into contact with one mollusc. Genetic exchanges were assessed from a "progeny-array analysis" by comparing the mother's genotype to that of her progeny at the rediae stage (larval stage in the first intermediate host). Electrophoretic analyses were performed according to the procedures described in Trouvé et al. (1996). The Em individuals showed a mixed mating system involving both selfing and outcrossing. Although variable (range 20–100%), the intraisolate outcrossing rates were very high since the total proportion of outcrossed offspring was 77% (Table 1).

Whereas our previous experiments on selfing rates (Trouvé et al. 1996) involved isolates originating from Egypt (El) and Madagascar (Ec) (interisolate situations), the present work was done on the Mali isolate for which we did not find any morphological or electrophoretic differences from the Egyptian one. In interisolate situations, the echinostomes reproduce by both self-fertilization and cross-fertilization (Table 1); the global outcrossing rate totaled 74% (Trouvé et al. 1996). The data were excluded when both individuals totally selfed (pair 5), as this could have been caused by no meeting and pairing between the mates in the host habitat.

We compared each intraisolate selfing rate to the interisolate estimates with Fisher's exact test (Sokal and Rolf 1981), using the NPstat program, and then with the sequential Bonferroni method (Rice 1989). There was no obvious difference in outcrossing rates (Table 1); also, the global statistic of the intra (mean 77%) and inter (mean 74%) outcrossing rates did not show significant p values according to Fisher's method ($p = .44$).

These results pointed out a predominance of cross-fertilization compared to self-fertilization in both inter- and intraisolate matings. However, in both cases the echinostomes partly self-fertilized. This suggests that selfing is certainly a reproductive mode used to ensure reproduction in the echinostomes. Otherwise the low probability of meeting a partner due to low mobility or population density (Charnov et al. 1976; Ghiselin 1974; Tomlinson 1966) could result in no reproduction. Furthermore, selfing is promoted by the cost of outcrossing, which refers to

Table 1. Comparison of intraisolate and interisolate outcrossing rates

Pair no.	Parent	Number of offspring analyzed	Number (%) of outcrossed offspring[a]	Pair no.	Parent	Number of offspring analyzed	Number (%) of outcrossed offspring[b]
1	Em1	13	10 (77)	1	Ec	19	11 (58)
	Em2	20	15 (75)		El	22	22 (100)
2	Em1	10	2 (20)	2	Ec	17	7 (41)
	Em2	15	4 (27)		El	24	8 (33)
3	Em1	16	11 (69)	3	Ec	21	16 (76)
	Em2	16	14 (87.5)		El	26	24 (92)
4	Em1	17	15 (88)	4	Ec	18	12 (67)
	Em2	20	17 (85)		El	25	11 (44)
5	Em1	16	10 (62.5)	5	Ec	20	0 (0)
	Em2	16	14 (87.5)		El	18	0 (0)
6	Em1	21	14 (67)	6	Ec	22	18 (82)
	Em2	19	18 (95)		El	18	18 (100)
7	Em1	24	22 (92)	7	Ec	20	20 (100)
	Em2	23	13 (56.5)		El	18	16 (89)
8	Em1	19	15 (79)				
	Em2	19	13 (68)				
9	Em1	24	10 (42)				
	Em2	24	20 (83)				
10	Em1	23	23 (100)				
	Em2	20	20 (100)				
11	Em1	18	16 (89)				
	Em2	18	14 (78)				
12	Em1	25	20 (80)				
	Em2	17	9 (53)				
13	Em1	26	22 (85)				
	Em2	26	20 (77)				
14	Em1	21	19 (90)				
	Em2	19	11 (58)				
15	Em1	18	5 (28)				
	Em2	23	23 (100)				
16	Em1	22	22 (100)				
	Em2	17	17 (100)				
17	Em1	17	12 (71)				
	Em2	17	16 (94)				
18	Em1	14	13 (93)				
	Em2	20	18 (90)				
19	Em1	25	23 (92)				
	Em2	26	12 (46)				
20	Em1	21	16 (76)				
	Em2	20	20 (100)				
Mean	**Em (Em1 and Em2)**	**19.6**	**15.2 (77)**	**Mean**	**Ec El**	**19.5 22.2**	**14 (72) 16.5 (74)**

Em1, Em2: two electrophoretic forms of the isolate of Mali; Ec, El: isolates originating from Madagascar and Egypt, respectively (see text).

[a] This study.

[b] Trouvé et al. (1996).

the transmission of genes and results from the doubling of parent-offspring relatedness under selfing compared to outcrossing. In this context, alleles have a 50% transmission advantage (Charlesworth 1980). On the other hand, the homozygosity resulting from selfing uncovers a genetic load by the expression of recessive deleterious mutations, which should promote random mating (Charlesworth and Charlesworth 1987). A mixed mating system can be selected as a consequence of biparental inbreeding (Ronfort and Couvet 1995; Uyenoyama 1986), a phenomenon that is expected in these organisms because the asexual reproduction at the larval stage may give rise to clonal individuals in the same final host. Such a mixed mating system has already been identified in pulmonate snails (Städler et al. 1993, 1995; Doums et al. 1996; Coutellec-Vreto et al. 1997; Viard et al. 1997) as well as in numerous plant species [Charlesworth and Charlesworth (1987) for a review; Karron et al. 1995].

Mate Choice

The choice of mate was analyzed by performing concurrent infections of mice with one Ek, one Em1, and one Em2 worm. The estimation of genetic exchanges was done following the same procedure as described above. For each replicate, the intraisolate outcrossing rate was compared with the interisolate one. Since these two datasets are not independent, we performed a sign test using Statistica 4.1 for Macintosh.

Three reproductive modes have been used by the Em individuals (Em1 and Em2): selfing, outcrossing with a related individual (intraisolate outcrossing), and outcrossing with an unrelated individual (interisolate outcrossing) (Table 2). However, Em individuals produced significantly more offspring through intraisolate outcrosses than via interisolate outcrosses ($p < .01$). This result is clearly illustrated by comparing the mean proportion of intra-outcrossed offspring (83%) with the mean of interoutcrossed offspring (7%) for Em individuals. This clearly highlights that individuals originating from the same isolate (Em) preferentially outcrossed with each other.

These results can be explained in view of the long process of two evolutionary factors: first, inbreeding depression is expected to decrease selfing rate and, second, hybrid breakdown likely reduces the interisolate outcrossing. These two factors act in synergy and seem to account for the high intra-cross-fertilization observed. Whereas this result on mate choice could be interpreted as postzygotic incompatibility between the two isolates, we should note that if such an incompatibility exists, it would have induced a decrease of the interisolate outcrossing rate in the first experiment with double infection. On the contrary, we did not observe a significant difference between intra- and interisolate outcrossing rates (Table 1). Although the mate choice experiment involved a small number of replicates, the results are highly significant and clearly confirm the hypothesis proposed in a previous article (Trouvé et al. 1996), namely, that there is mate preference between related individuals.

This preferential intraisolate outcrossing may result from two phenomena: (1) a preferential mating between related individuals, that is, assortative mating. Such a phenomenon has already been pointed out in a number of species, for example, *Drosophila melanogaster (*Wu et al. 1995), *Tribolium confusum (*Wade et al. 1995), as well as in parasites, on schistosomes (Tchuem Tchuenté et al. 1993), or (2) a postcopulatory prezygotic isolation, that is, a gametic selection which would operate in the female genital tract (sperm competition or cryptic female choice; Eberhard 1996). These postcopulatory isolation mechanisms have been observed in a wide range of taxa [for reviews see Bishop (1996), Bishop et al. (1996), Gomendio and Roldan (1993), Parker (1990), and Wirtz (1997)]; however, no study has been carried out in this context for parasites.

Table 2. Selfing and outcrossing rates exhibited by each parent of a group of three worms: Em1 + Em2 + Ek

Trio no.	Parent	Number of offspring analyzed	Number (%) of eggs fertilized by the sperm of		
			Em1	Em2	Ek
1	Em1	14	0 (0)s	14 (100)	0 (0)
	Em2	16	12 (75)	3 (19)s	1 (6)
	Ek	17	9 (53)	8 (47)	0 (0)s
2	Em1	23	6 (26)s	14 (61)	3 (13)
	Em2	19	19 (100)	0 (0)s	0 (0)
	Ek	17	16 (94)	0 (0)	1 (6)s
3	Em1	24	0 (0)s	24 (100)	0 (0)
	Em2	20	14 (70)	2 (10)s	4 (20)
	Ek	17	2 (12)	15 (88)	0 (0)s
4	Em1	20	6 (30)s	14 (70)	0 (0)
	Em2	19	16 (84)	0 (0)s	3 (16)
	Ek	21	10 (48)	11 (52)	0 (0)s
5	Em1	21	0 (0)s	21 (100)	0 (0)
	Em2	21	16 (76)	3 (14)s	2 (10)
	Ek	19	5 (26)	12 (63)	2 (11)s
Mean	**Em1**	**20.4**	**2.4 (12)s**	**17.4 (85)**	**0.6 (3)**
	Em2	**19**	**15.4 (81)**	**1.6 (8)s**	**2 (11)**
	Ek	**18.2**	**8.4 (46)**	**9.2 (51)**	**0.6 (3)s**

Em1, Em2: two electrophoretic forms of the isolate of Mali; Ek: isolate originating from Cameroon (see text); s = selfing rate.

Hence further work is needed to distinguish between these hypotheses.

From the Centre de Biologie et d'Ecologie Tropicale et Méditerranéenne, Laboratoire de Biologie Animale, Université de Perpignan, Perpignan, France (Trouvé, Durand, and Jourdane), and Centre d'Etude sur le Polymorphisme des Micro-Organismes, CEPM/UMR CNRS-IRD, Equipe: "Evolution des Systèmes Symbiotiques," Montpellier, France (Renaud). Address correspondence to Sandrine Trouvé, Institut de Zoologie et d'Ecologie Animale, Bâtiment de Biologie, Université de Lausanne, 1015 Lausanne, Switzerland, or e-mail: sandrine.trouve@ie-zea.unil.ch. The authors would like to thank M.-T. Almeras, B. Dejean, and P. Pasquereau for excellent technical assistance. We also wish to acknowledge J. M. Greeff, T. de Meeüs, and T. Städler for helpful comments on the first draft of this manuscript. We are also very grateful to K. L. Carter for constructive suggestions which greatly improved the final form of the manuscript. We address special thanks to A. Dabo for collecting the animals in Mali. This work was supported by the CNRS (Sciences de la Vie) and by the "Ministère de l'Enseignement Supérieur et de la Recherche" by providing a grant to S.T. This manuscript was written while S.T. was in receipt of a grant from the "Fondation Robert Schuman" which we would like to thank.

References

Bishop JDD, 1996. Female control of paternity in the internally fertilizing compound ascidian *Diplosoma listerianum.* I. Autoradiographic investigation of sperm movements in the female reproductive tract. Proc R Soc Lond B 263:369–376.

Bishop JDD, Jones CS, and Noble LR, 1996. Female control of paternity in the internally fertilizing compound ascidian *Diplosoma listerianum.* II. Investigation of male mating success using RAPD markers. Proc R Soc Lond B 263:401–407.

Charlesworth B, 1980. The cost of sex in relation to mating system. J Theor Biol 84:655–671.

Charlesworth D and Charlesworth B, 1987. Inbreeding depression and its evolutionary consequences. Annu Rev Ecol Syst 18:237–268.

Charnov EL, Maynard Smyth J, and Bull JJ, 1976. Why be an hermaphrodite? Nature 263:125–126.

Coutellec-Vreto MA, Madec L, and Guiller A, 1997. Selfing and biparental inbreeding: a mating system analysis in *Lymnaea peregra* (Gastropoda: Lymnaeidae). Heredity 79:277–285.

Doums C, Viard F, Pernot AF, Delay B, and Jarne P, 1996. Inbreeding depression, neutral polymorphism and copulatory behavior in freshwater snails: a self-fertilization syndrome. Evolution 50:1908–1918.

Eberhard WG, 1996. Female control: sexual selection by cryptic female choice. Princeton, NJ: Princeton University Press.

Ghiselin MT, 1974. The economy of nature and the evolution of sex. Berkeley: University of California Press.

Gomendio M and Roldan ERS, 1993. Mechanisms of sperm competition: linking physiology and behavioural ecology. Trends Ecol Evol 8:95–100.

Jarne P, 1995. Mating system, bottlenecks and genetic polymorphism in hermaphroditic animals. Genet Res 65:193–207.

Jarne P, Vianey-Liaud M, and Delay B, 1993. Selfing and outcrossing in hermaphrodite freshwater gastropods (Basommatophora): where, when and why. Biol J Linn Soc 49:99–125.

Karron JD, Thumser NN, Tucker R, and Hessenauer AJ, 1995. The influence of population density on outcrossing rates in *Mimulus ringens.* Heredity 75:175–180.

Michiels NK and Streng A, 1998. Sperm exchange in a simultaneous hermaphrodite. Behav Ecol Sociobiol 42: 171–178.

Parker GA, 1990. Sperm competition: raffles and roles. Proc R Soc Lond B 242:120–126.

Rice WR. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.

Ronfort J and Couvet D, 1995. A stochastic model of selection on selfing rates in structured populations. Genet Res 65:209–222.

Sokal RR and Rohlf FJ, 1981. Biometry, 2nd ed. New York: W.H. Freeman.

Städler T, Loew M, and Streit B, 1993. Genetic evidence for low outcrossing rates in polyploid freshwater snails (*Ancylus fluviatilis*). Proc R Soc Lond B 251:207–213.

Städler T, Weisner S, and Streit B, 1995. Outcrossing rates and correlated matings in a predominantly selfing freshwater snail. Proc R Soc Lond B 262:119–125.

Tchuem Tchuenté LA, Imbert-Establet D, Delay B, and Jourdane J, 1993. Choice of mate, a reproductive isolating mechanism between *Schistosoma mansoni* in mixed infections. Int J Parasitol 23:179–185.

Tomlinson J, 1966. The advantages of hermaphroditism and parthenogenesis. J Theor Biol 11:54–58.

Trouvé S and Coustau C, 1998. Differences in excretory-secretory products from adult echinostomes of related species. J Parasitol 84:1062–1065.

Trouvé S, Renaud F, Durand P, and Jourdane J, 1996. Selfing and outcrossing in a parasitic helminth (Trematoda, Echinostomatidae). Heredity 77:1–8.

Trouvé S, Renaud F, Durand P, and Jourdane J, 1998. Experimental evidence of hybrid breakdown between genetically distinct populations of *Echinostoma caproni.* Parasitology 117:133–135.

Uyenoyama MK, 1986. Inbreeding and the cost of meiosis: the evolution of selfing in populations practicing biparental inbreeding. Evolution 40:388–404.

Viard F, Doums C, and Jarne P, 1997. Selfing, sexual polymorphism and microsatellites in the hermaphroditic freshwater snail *Bulinus truncatus.* Proc R Soc Lond B 264:39–44.

Wade MJ, Chang NW, and McNaughton M, 1995. Incipient speciation in the flour beetle *Tribolium confusum*: premating isolation between natural populations. Heredity 75:453–459.

Wirtz P, 1997. Sperm selection by females. Trends Ecol Evol 12:172–173.

Wu C-I, Hollocher H, Begun DJ, Aquadro CF, Xu Y, and Wu ML, 1995. Sexual isolation in *Drosophila melanogaster*: a possible case of incipient speciation. Proc Natl Acad Sci USA 92:2519–2523.

Received September 14, 1998
Accepted May 31, 1999

Corresponding Editor: Martin Tracey

Quantitative Trait Loci Influencing Honeybee Alarm Pheromone Levels

G. J. Hunt, A. M. Collins, R. Rivera, R. E. Page Jr., and E. Guzmán-Novoa

Quantitative trait loci (QTL) mapping procedures were used to identify loci that influence the levels of alarm pheromones found in the stinging apparatus of worker honeybees. An F_1 queen was produced from a cross between a queen of European origin and a drone descended from an African subspecies. Haploid drones from the hybrid queen were individually backcrossed to European queens to produce 172 colonies. Samples of stings were taken from backcross workers of these colonies. Alarm pheromone levels were determined by gas chromatography. RAPD markers were scored from the haploid drone fathers of these colonies. The multiple-QTL model (MQM) of MapQTL was used to identify QTLs that influence the levels of four alarm pheromone components. Seven independent, potential QTLs were identified with LOD scores greater than two, and one at LOD 1.88. We identified one QTL for n-decyl acetate,