Inbreeding in the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae) estimated from endosulfan resistance phenotype frequencies

D. Pierre Gingerich

Section of Ecology and Systematics, Cornell University, Ithaca, New York, USA

Philippe Borsa*

ORSTOM, Institut Français de Recherche Scientifique pour le Développement en Coopération, Nouméa, New Caledonia

D. Maxwell Suckling

Horticulture and Food Research Institute of New Zealand Ltd., Lincoln, Christchurch, New Zealand

Luc-Olivier Brun

ORSTOM, Institut Français de Recherche Scientifique pour le Développement en Coopération, Nouméa, New Caledonia

Abstract

An estimate of the inbreeding coefficient, F_{IS}, of the coffee berry borer, *Hypothenemus hampei* Ferrari, was calculated from genotype frequencies of endosulfan resistance in beetles collected from berries in 41 different fields on the East Coast of New Caledonia. Two different estimates were obtained as a function of sampling date: $\hat{F}_{IS}=0.491\pm0.059$ (s.e.) for samples collected in September, and $\hat{F}_{IS}=0.215\pm0.108$ for samples collected in April. These values of $\hat{F}_{IS}$ are very high in comparison to those of most insects, but surprisingly low given current understandings of *H. hampei* mating patterns. The difference between April and September F_{IS} estimates is discussed in terms of insecticide regimes and seasonal variability of sib inbreeding levels. The high level of inbreeding in the coffee berry borer increases frequencies of homozygotes relative to heterozygotes. Inbreeding will accelerate resistance evolution whenever the fitness of homozygous resistant insects exceeds that of heterozygotes, and will undermine any high-dose strategy to control resistance evolution.

Correspondence: D.P. Gingerich, Section of Ecology and Systematics, Corson Hall, Cornell University, Ithaca, New York 14853, USA.
*Present address: ORSTOM UR 33-Zoologie, B.P. 5045, 34032 Montpellier cedex 1, France.

Introduction

Hypothenemus hampei Ferrari (Coleoptera: Scolytidae), the coffee berry borer (CBB), a scolytid beetle of African origin, is now the most important insect pest of coffee throughout the tropics. The CBB belongs to one of at least seven scolytid lineages that have independently evolved sib breeding. These are nearly all characterized by a suite of shared traits: a female-biased sex ratio, pre-dispersal mating, female-initiated colonization, and dwarfed males (Kirkendall, 1993). CBB females outnumber males ten to one (Corbett, 1933; Bergamin, 1943); such skewing is evolutionarily advantageous in organisms where brothers compete for mates (Hamilton, 1967). Only females can colonize new berries. Males, much smaller than females and incapable of flight, play at most a minor role in dispersal. Females are thus presumed to mate with their brothers before dispersal.

Hypothenemus hampei is pseudo-arrhenotokous; although males receive paternal chromosomes, only the maternally derived resistance allele is expressed in males (Brun *et al.*, 1995b) and transmitted through males to progeny (Brun *et al.*, 1995a; Borsa & Kjellberg, 1996). Eggs produced by unmated *H. hampei* females are few in number and nonviable (Hargreaves, 1926; Bergamin, 1943; Giordanengo, 1992; but see Muñoz, 1989).

F-statistics measure the effects of inbreeding and population subdivision on genotype frequencies. Wright (1969) uses F_{IS} and F_{ST} respectively to measure population structure within and between subpopulations, the smallest scale of a hierarchical sampling scheme (here represented by single samples). For selectively neutral traits, F_{IS} corresponds to the proportion by which heterozygosity falls short of its predicted frequency under Hardy-Weinberg equilibrium assumptions. F_{IS}-values of 0 and 1 correspond to panmixis and complete inbreeding, respectively.

Populations of the CBB in New Caledonia (South Pacific) have developed resistance to endosulfan, the principal insecticide used for its control (Brun *et al.*, 1989). Endosulfan resistance, the genetic trait best characterized in the CBB, may be largely accounted for by a point mutation in the *Rdl* gene, which encodes a GABA$_A$-receptor subunit (ffrench-Constant *et al.*, 1994). In laboratory bioassays, the expression of the susceptible (S) and resistant (R) alleles of *Rdl* is codominant (Brun *et al.*, 1995b). Thus bioassays at two doses can be used to obtain estimates of frequencies of all three resistance genotypes (SS, RS, RR) and of F_{IS} for each of multiple field-collected samples. The possible confounding effects of selection on F_{IS}-values are discussed below.

The F_{IS}-values presented contribute to the understanding both of insecticide resistance evolution and scolytid mating systems. By increasing homozygosity in populations, inbreeding increases the fitness advantage (and hence the rate of evolution) of less-than-completely dominant resistance alleles in populations under selection pressure, a factor not yet examined in models of insecticide resistance evolution. Models of resistance evolution based on actual field data generally assume Hardy-Weinberg equilibrium frequencies in extrapolating genotype frequencies from bioassays at a single dosage. This paper documents a case where this assumption is not valid, quantifies inbreeding, and examines some of its implications for the interpretation of data on resistance development in CBB populations in New Caledonia.

The Scolytidae are characterized by diverse breeding behaviours and sex determination systems (Kirkendall, 1993). By providing estimates for the amount of sib-mating behaviour in this scolytid species, this study may contribute to understanding of the patterns and constraints of evolution of inbreeding in this large and economically important family.

Materials and methods

Sampling

CBB females were extracted from 48 samples of infested berries collected from 41 fields in New Caledonia in 1991. Samples were collected from nine fields in April and from 39 fields in September, of which seven had been sampled in April. Fields varied with respect to the type of cultivation, the geographic region, and the insecticide treatment.

Fifteen samples were from traditional fields, composed of old, unpruned, semi-abandoned plants under dense shade; 33 samples were from modern fields without shade, with relatively small bushes under ten years old. Samples were gathered from four regions on the East Coast: Touho (21 samples), Ponérihouen (18), Poindimié (7), and Hienghène (2).

All nine April samples were from fields in the Touho region treated with endosulfan. Samples collected in September were from fields with one of three treatments: 14 with endosulfan; 23 with fenitrothion, an organophosphate showing no cross-resistance with endosulfan (Brun *et al.*, 1994) and for which no field resistance has been detected; and 2 untreated. Since insecticide treatments were applied by the agricultural development agency of New Caledonia (ADRAF) for purposes of pest control rather than experimental design, treatment and region are confounded: all sites in Touho and Hienghène were treated with endosulfan, while all those in Poindimié and Ponérihouen were treated with fenitrothion or untreated.

All treated fields were sprayed in January or early February. In New Caledonian coffee fields, insecticides are applied from truck-mounted sprayers confined to field margins, and have little effect beyond 30 metres (Parkin *et al.*, 1992). Infested berries were gathered across the width of fields, and collection was limited to field edges, along which insecticide treatment was fairly constant.

Bioassays

Because males are rare and show only two distinguishable resistance phenotypes (Brun *et al.*, 1995b; Borsa & Kjellberg, 1996), only females were systematically examined. Two subsamples of CBB females were extracted from each berry sample, and bioassayed with endosulfan, using a direct spray technique described by Brun *et al.* (1989). One subsample was tested at one of two high dosages; the other subsample at a low dosage.

Putative RR insects were discriminated from putative RS and SS insects by a bioassay at either of two high dosages: HighDose1=25,000 ppm, with mortality evaluated at 6 h; and HighDose2=2500 ppm, with mortality evaluated at 7 days on insects transferred to artificial diet 6 h after treatment. All nine April samples were tested at HighDose2; In September, HighDose1 and 2 were used to test 20 and 19 samples, respectively. Brun *et al.* (1995b) found that both

high dosages cause 100% mortality of field-collected susceptible strains ('SS'), >98% mortality in 'RS' F1 hybrid individuals bred in the laboratory, and 5% mortality in highly selected resistant laboratory strains ('RR'). The low dosage was 400 ppm endosulfan, scored at 6 h. Treatment at the low dosage, chosen for diagnosis of resistance at the $LD_{99.95}$ of susceptibles (Brun *et al.*, 1989), causes death in 23% of 'RS' females (Brun *et al.*, 1995b).

The insect samples, each divided into two roughly equal subsamples, ranged in size from 45–185 insects, averaging 158 ± 40 (s.d.) insects. Beetles were allowed to develop in the laboratory within berries for several weeks so that a greater number of mature adults would be available for testing. Multiple insects per berry (often sisters) were frequently recovered and tested together. Sampling of sisters together is expected to have increased the variance of $\hat{F}_{IS}$; however, it should not have appreciably biased estimates, since individuals tested were the offspring of matings in the field, not the laboratory.

Statistical methods

The proportions of CBB females from sample i belonging to each putative genotype were calculated as follows: To a first approximation, $[\tilde{R}R]_i$ equals the proportion of insects surviving the high dosage treatment; $[\tilde{S}S]_i$ the mortality at the low dosage, and $[\tilde{R}S]_i = 1 - [\tilde{R}R]_i - [\tilde{S}S]_i$. The genotype proportions were then adjusted to take into account the proportion of putative RS (23%) suffering mortality at the low dosage, as well as minor control mortality of the 'RR' and control survival of 'RS' at the high dosages.

Given that putative RS insects experience substantial mortality at the low dosage, estimates of genotype proportions will suffer from two types of bioassay uncertainty in addition to variance at the resistance locus in the samples of insects. First, if each of n RS individuals tested at the low dosage dies with independent probability p, then total RS mortality will follow a binomial distribution with variance $p(1-p)n$, and the RS kill rate for the sample will have variance $p(1-p)/n$. This binomial variance increases as p departs from zero. Second, minimum lethal dosages for individuals from homogeneous populations generally follow a log-normal distribution; the closer a dosage is to the centre of a mortality curve, the greater will be the extra-binomial variance in mortality provoked by small perturbations in test conditions.

An estimate of F_{IS} was calculated for each sample as $\hat{F}_{IS}(i) = 1 - [\tilde{R}S]_i / 2\tilde{p}_i\tilde{q}_i$, where $\tilde{p}_i$ and $\tilde{q}_i$ are sample-specific allele frequency estimates: $\tilde{p}_i = [\tilde{S}S]_i + [\tilde{R}S]_i / 2$ and $\tilde{q}_i = 1 - \tilde{p}_i$. Since values of $\hat{F}_{IS}(i)$ become extremely sensitive to even slight bioassay problems as $\tilde{p}_i$ values approach 0 or 1, samples for which $\tilde{p}_i$ or $\tilde{q}_i$ was less than 6% were excluded from the data set. Further, each sample was weighted by $w_i = \tilde{p}_i\tilde{q}_i\tilde{n}_i$, the reciprocal of an estimate of the sampling variance of $\hat{F}_{IS}(i)$, where $\tilde{n}_i$ is the harmonic mean of the two subsample sizes for each sample (Nei, 1977; Weir & Cockerham, 1984). Averages and their variances determined across k samples were estimated as follows:

$$\hat{F}_{IS} = \frac{\Sigma w_i \hat{F}_{IS}(i)}{\Sigma w_i}, \text{ and } Var(\hat{F}_{IS}) = \left(\frac{\Sigma w_i \hat{F}_{IS}(i)^2}{\Sigma w_i} - \hat{F}_{IS}^2\right)\left(\frac{1}{k-1}\right)$$

ANOVAs (again based on weighted data) were employed for all comparisons of $\hat{F}_{IS}$ between treatments. Whenever only two treatment levels are compared, the test of Snedecor's F-statistic derived from an ANOVA is formally equivalent to a two-sided t-test. Given the weighting of data, the ANOVA was computationally more manageable.

The particular form of the data set, genotype frequencies estimated from bioassays on two independent subsamples per sample, is to our knowledge unprecedented in the inbreeding literature. Thus modelling was undertaken to detect any statistical bias (i.e. systematic difference between parameter values and their estimates) introduced by use of a ratio of two estimates ($[\tilde{R}S]_i$ and $\tilde{p}_i\tilde{q}_i$), or by the data selection criterion and weighting scheme. The expectation of $\hat{F}_{IS}(i)$ was calculated for a hypothetical field with known parameter values, p and $F_{IS}(i)$. This expectation is the average of the $F_{IS}(i)$-values calculated as above for all of the possible bioassay outcomes for samples, with each term weighted as above, and also by the probability of that outcome, given the parameter values. The whole procedure was repeated over a wide range of parameter values. Our method of calculation was found to underestimate the parameter, $F_{IS}(i)$, by a small factor well approximated by $0.020(1 - \hat{F}_{IS}(i))$. This correction factor was calculated for each field and applied to obtain the revised $\hat{F}_{IS}(i)$ values used for calculating summary and comparison statistics (as above).

Results

Levels of resistance (putative RS + RR) in the samples studied were 0.164 ± 0.073 (s.d.) in Poindimié, 0.356 ± 0.217 in Ponérihouen, 0.405 ± 0.010 in Hienghène, and 0.676 ± 0.286 in Touho. Resistance levels remained unchanged between April and September samplings in Touho.

The F_{IS}-estimate (± s.e.) calculated across the entire data set was 0.461 ± 0.053 (n = 48). Weighted means of $\hat{F}_{IS}$-values for each level of sampling date, insecticide treatment, field cultivation type, and HighDose, are presented in table 1. The degrees of freedom, test statistic, and p-value for ANOVAs comparing F_{IS}-estimates taken across different data subsets are presented in table 2. When F_{IS}-values for the nine April samples (mean 0.215 ± 0.108 (s.e.)) are compared with those from the 39 September samples (0.491 ± 0.059), the difference is suggestive, but not significant ($P = 0.102$). The same result is obtained when the April samples are compared only with the fourteen September samples from endosulfan-treated fields ($P = 0.097$). However, a clearer contrast is provided by a paired comparison of April and September samples from the seven fields sampled at both times ($P = 0.043$).

The comparison of F_{IS}-values by insecticide treatment, limited to September samples because all April samples were from fields treated alike with endosulfan, did not show any significant effect. Given evidence from Brun & Suckling (1992) that field cultivation type has an effect on insecticide efficacy in the field, we might have expected differences in F_{IS} due to field type at the April sampling. Any such effect, however, was well below a threshold of significance, as was the contrast by field type for endosulfan-treated fields sampled in September, as well as the contrast by field type over all fields studied.

Table 1. F_{IS}-estimates and sample sizes for factor levels.

Factor	Level	n	F_{IS}-estimate (mean ± s.e.)
All samples		48	0.461 ± 0.053
Date of sampling	September	39	0.491 ± 0.059
	April (Trt=endosulfan)	9	0.215 ± 0.108
Treatment (Sept. only)	endosulfan	14	0.546 ± 0.109
	fenitrothion	23	0.474 ± 0.075
	no treatment	2	0.331 ± 0.009
Field type	sunny	33	0.492 ± 0.065
	shady	15	0.381 ± 0.094
HighDose (Sept. only)	1) 25,000 ppm/6 h	20	0.569 ± 0.093
	2) 2500 ppm/7 d	19	0.416 ± 0.069

Means of estimates of the inbreeding coefficient ($\hat{F}_{IS}$), calculated across the entire data set and for each level of sampling date, insecticide treatment, field cultivation type, and RR/RS discriminating dose. Data were weighted as described in the text. All April samples were tested at HighDose2 and came from fields treated with endosulfan; only September data were included for calculation of $\hat{F}_{IS}$ for the insecticide treatment and HighDose levels.

Table 2. Comparisons of $\hat{F}_{IS}$ broken down by single factors.

Factor	Data used	d.f.	Snedecor's F-statistic	Prob.
Date of sampling	All	1, 46	2.791	**0.102**
	Trt=endosulfan	1, 21	3.024	**0.097**
	Paired fields	1, 6	6.559	**0.043**
Insecticide trt.	All	2, 45	0.200	0.820
	Date=Sept.	2, 36	0.452	0.640
Field type	All	1, 46	0.880	0.353
	Date=April	1, 7	0.309	0.596
	Date=Sept.	1, 37	1.478	0.232
Geographic region	Date=Sept.	3, 35	1.804	0.165
HighDose	Date=Sept.	1, 37	1.746	0.195

ANOVA test statistics, degrees of freedom, and probability of observed outcome given H_0 (no difference between levels) are given for $\hat{F}_{IS}$ tested on single factors. Data were weighted as described in the text. Where the analysis employed only a portion of the entire data set, this is indicated under 'Data used'.

Finally, the choice of the two dosages employed for the high-dosage bioassay had no significant effect on the F_{IS}-estimates, as expected for these ostensibly equivalent bioassays.

Discussion

CBB population and breeding structure

F_{IS}-values obtained in this study are similar to those obtained by Borsa & Gingerich (1995) in a preliminary study of allozymes of *H. hampei* from New Caledonia and the Ivory Coast. In that study, allozyme variation at two presumably neutral loci, *Mdh*-2 and *Mpi*, characterized in 88 and 21 insects over 7 and 4 fields, respectively, allowed an overall field-scale F_{IS} estimate of 0.273 ± 0.134 (s.e.). However, the form of those data did not permit analysis of seasonal trends in F_{IS}.

In this study, F_{IS}-statistics were calculated from genotype frequencies of resistance, a trait periodically under strong selection. Because annual insecticide treatments affect the levels of endosulfan resistance in fields, the standard assumption that population differentiation between fields is at an equilibrium determined by opposing forces of genetic drift and migration does not apply. These data thus provide no basis for inference on population structure at scales larger than the field. Within each field, however, F_{IS}-values are expected to return rapidly to equilibrial levels following treatment, and thus provide useful information on population structure within the field (see below). The sample of insects from each field was treated as an independent replicate; migration between fields was ignored. The scale of between-field migration and thus its role in inflating $\hat{F}_{IS}$ are probably much less important than those of within-field migration (discussed below). A single sampling scale was examined – the field or, more precisely, the field edge, over which conditions were assumed to be relatively homo-

geneous. Release-recapture studies in three fields (Gingerich, 1996) showed single-generation movement (i.e. dispersal from a point source to berries colonized) averaging from 4 to 13 metres, with some females dispersing to (and presumably beyond) the edges of the fields (>30 metres). This degree of within-field insect movement appears sufficient to prevent the development of differences in allele frequencies across the sampled area. Thus nearly all depression of heterozygote levels can reasonably be attributed to incestuous breeding.

A constant inbreeding regime over multiple generations will drive F_{IS}-values toward an equilibrium ($F_{IS}*$). In the simplest model, a proportion, C, of matings every generation is with unrelated individuals, and all inbreeding is by mating between sibs. In this case, $F_{IS}*=(1-C)/(1+3C)$, or inversely, $C=(1-F_{IS}*)/(1+3F_{IS}*)$ (Falconer, 1989). If $F_{IS}*$ is set to April and September values of $\hat{F}_{IS}$ (0.215 and 0.491, respectively), C equals 47.7% and 20.6%. Additional clutches are frequently observed within berries (Giordanengo, 1992); however, the relative proportions of these insects which are offspring of the original colonizing female and of her daughters are unclear. Any shift of model assumptions to allow some members of a second generation of offspring to mature and interbreed within berries would require higher outcrossing to generate a given value of $F_{IS}*$. Given that inbreeding appears to be the norm in this species, two possible mechanisms are considered which may account for the surprisingly high outcrossing observed: (i) mating during or after initial dispersal; and (ii) multiple infestations of given berries.

Females emerging on their own from their natal berries showed >99% fecundity (no sample sizes given) when placed on fresh berries (Bergàmin, 1943; Giordanengo, 1992). Similarly, 95% of 512 females emerging from interseason berries in the laboratory produced viable offspring on artificial medium (Mathieu *et al.*, 1993), likely an underestimate of the proportion actually mated. Likewise, 90% (*n*=124) of flying females captured in the field with non-destructive olfactory traps produced viable eggs on artificial medium (F. Mathieu & L.O. Brun, in preparation), an indication that pre-mating flights are rare or non-existent under field conditions. Finally, Giordanengo (1992) observed that no virgin adult females (*n*=64) flew under laboratory test conditions that caused 64% (*n*=300) of mated females to fly. These studies strongly suggest that few females disperse before mating (but see López & Frérot, 1993). However, they do not rule out the possibility that virgin females seek out mates by walking; in the laboratory studies by Giordanengo and by Mathieu *et al.*, females came from berries grouped together in darkened emergence boxes, and some females may have walked between berries to mate before 'emergence'. The conditions of the Bergamin study are unclear in this respect. Although the possibility of dispersal by walking of unmated females deserves further examination, it seems unlikely that post-emergence mating alone could account for even 20.6% outcrossing (calculated above for September), assuming females mate only once. Dispersal of males by walking is probably insignificant; Giordanengo (1992) observed that of 1201 insects emerging from field-infested berries placed in emergence boxes, only 3 were males. In species such as *H. hampei*, which do not have the option of parthenogenesis, pre-dispersal mating could serve as cheap 'fecundity insurance' for a female, assuring her reproduction even if she should prove unable

to find an unrelated mate (Kirkendall, 1993). In the Ivory Coast, Ticheler (1961) observed irreversible degeneration of flight muscles in all mated females that had become established in a berry. Provided this observation holds true for New Caledonian populations, then any possible outbreeding of already sib-mated females must occur before berry colonization or be limited to males that females can reach by walking. We are aware of no study examining sperm priority in *H. hampei*, or even the occurrence of multiple mating.

Coffee berries are most often infested by a single mated female, which produces about 50 offspring in a first clutch (Le Pelley, 1968). Since each female is said normally to bore her own hole (Giordanengo, 1992), colonization of a berry by more than one female should be detectable by multiple entrance holes (Giordanengo, 1992). Although multiple infestations seem fairly uncommon most of the year, they are prevalent during the coffee interseason (in New Caledonia roughly mid-December to March). By the end of the interseason, the few remaining dried berries on coffee bushes generally have multiple perforations, and are little more than hollow shells full of dozens of CBBs per berry. While it is not clear that the number of these perforations gives a good estimate of the number of colonizing females, it seems likely (though not demonstrated) that berries hold multiple females taking refuge as adults from harsh seasonal conditions, not simply the undispersed offspring of a single colonizing female. Although accumulation of large numbers of insects in berries with limited resources should require that adults maintain a sort of quiescent state, F. Mathieu & L.O. Brun (in preparation) have also observed some production of immature stages, which would likely outbreed once mature.

Temporal trends in $\hat{F}_{IS}$

The pattern of $\hat{F}_{IS}$ over sampling date in endosulfan-treated fields, paired with the lack of significance of field insecticide treatment in September, suggests two possibly complementary hypotheses: (i) sampling date effects reflect annual fluctuations in breeding patterns; and (ii) perturbations in resistance allele frequencies generated by strong selection in January are present in April but attenuated by September.

Gingerich (1996) modelled the effects on F_{IS}-values when a constant low level of outbreeding (20–30%) is interrupted by one or more generations of high outbreeding (80–100%). Even a single generation of complete outbreeding will lower F_{IS}-values in the offspring to zero. When inbreeding returns to its original higher levels, F_{IS}-values only gradually return to equilibrium, with the perturbation diminishing by 30–40% each generation, or initially somewhat faster if the breeding regime is altered during only one generation. CBB in New Caledonia go through roughly five generations per year (Giordanengo, 1992). Thus the insects sampled in April are probably the first and second-generation offspring of CBBs which outbred during the coffee interseason, whereas the populations sampled in September will have had two additional generations to return toward a high inbreeding equilibrium. The observed reduction in F_{IS}-values in April compared to September is consistent with the hypothesis of a seasonal shift in inbreeding behaviour.

When fields are treated with endosulfan in January, affected SS insects experience near-total mortality, whereas field mortality of RS insects, though not well characterized, is probably under half as great. In contrast, analysis of resistance-level patterns in fields treated with fenitrothion or untreated controls reveals slight or no selection against the resistance allele (D.P. Gingerich, D.M. Sucking & L.O. Brun, in preparation). The disproportionate homozygote (SS) mortality under endosulfan treatment should shift $\hat{F}_{IS}$ well below equilibrium in the treated generation, though the effect should vary with allele levels. In the hypothetical case in which survivorship of treated RR, RS, and SS insects is 1, 0.8, and 0.1, and F_{IS} prior to selection is 0.4, the predicted value of F_{IS} immediately after selection is roughly zero for pre-selection R-allele frequencies above 0.2 (corresponding to post-selection R-allele frequencies above about 0.6). For lower resistance levels, smaller depressions of F_{IS} are observed. Either lower SS mortality or increased RS mortality would cause smaller reductions in F_{IS}-values following selection.

Since endosulfan applications reach only the border strip from which samples were taken (Parkin *et al.*, 1992), the interior of the field constitutes a reservoir of comparatively susceptible insects that could mix with the more resistant border population (Brun & Suckling, 1992). Gradients of increasing endosulfan resistance from field centre to edge have been observed in fields with recent endosulfan treatment (Brun & Suckling, 1992). Migration across such gradients should increase the measured $\hat{F}_{IS}$, since it results in sample units within which populations with divergent resistance frequencies are pooled (the Wahlund effect, see Hartl & Clark, 1989). This increase is roughly proportional to the proportion of migrants in the population and to the square of the difference of allele frequency between immigrant and resident populations.

Gingerich (1996) has modelled the pattern of allele frequencies following a single generation of strong selection, assuming that inbreeding behaviour remains unchanged and that there is no migration. While the precise trajectory of return of F_{IS} to equilibrium depends on the details of survivorship of both female and male genotypes, the initial perturbation in F_{IS} is reduced each generation by 30–50%. When untreated susceptibles from the back of the field can migrate into the treated front edge of the field, the return to high equilibrial F_{IS}-values is accelerated. In field populations with low resistance, the proportion of migrants resulting from even a small inflow is likely to be large immediately after treatment, given the decimation of the resident population. If the proportion of migrants is high (15–20%) then F_{IS}-values measured even one generation after selection are expected to be within 0.05 of equilibrium.

Models based on both hypotheses for seasonal difference in F_{IS}-values (fluctuations in breeding patterns or selection effects plus migration) demonstrate seasonal patterns in F_{IS}-statistics broadly consistent with the observed results: F_{IS}-values in treated fields in April (one or two generations after treatment) are lower than those in September (three or four generations after treatment). Quantitative characterizations of migration rates, actual selection rates in the field, and behavioural patterns associated with inbreeding are all insufficiently precise to allow an evaluation of the relative importance of these two factors. The lack in September of significant difference in F_{IS}-values across insecticide treatments indicates that by that time any residual

effects of selection in January are likely to be insignificant. Thus both field data and modelling results suggest that the F_{IS}-values derived from September sampling provide a good estimate of inbreeding levels of the CBB during seasons and conditions in which an abundance of berries is available.

Comparison with other insect systems

Kirkendall (1993) reviews studies on dozens of species from diverse genera of Scolytidae for which there is strong evidence of sib mating. However, this paper is the first study that attempts to quantify sib mating of scolytids using F_{IS}-statistics.

Published estimates of F_{IS} are generally ancillary to estimates of population differentiation, measured with F_{ST} (Prout & Barker, 1993). Several population studies have noted elevated F_{IS} values in Coleoptera: 0.430–0.450 in three troglodytic beetles of the genus *Speonomus* (Crouau-Roy, 1988), 0.33 in two leaf beetles, *Oreina cacaliae* Schrank and *O. speciosissima* Scopoli (Chrysomelidae) (Rowell-Rahier, 1992), and 0.349 in *Collops tricolor* Say (Melyridae) (King, 1988). Other studies have shown high F_{IS} values in nabids (Grasela & Steiner, 1993) and termites (Reilly, 1987). Non-zero F_{IS} levels may reflect inbreeding in the strict sense (e.g. between sibs, or between parents and offspring), or the Wahlund effect, or selection. Whereas most of these studies discuss the possibility of inbreeding but make no attempt to quantify it, Prout & Barker's (1993) study of inbreeding in *Drosophila buzzatii* Patterson & Wheeler (Diptera: Drosophiladae) explicitly examines the problems involved in inferring sib-breeding levels from F_{IS}-statistics determined from insects sampled from ephemeral (3-generation) colonies on *Opuntia*. In contrast to CBB, colony-founding *D. buzzatii* females are thought to outbreed after dispersal from their colony of origin. In both studies, however, uncertainty regarding the relative weight of dispersing and sampled generations means that F_{IS}-estimates can provide only bounds for inbreeding rates, not fixed values.

Importance of inbreeding for resistance transmission

Inbreeding must be considered as a factor in the evolution of insecticide resistance. Where measures of population structure have been incorporated in previous field studies and models of resistance evolution, the parameter examined has been F_{ST}, which reflects gene flow between populations (Caprio & Tabashnik, 1992a,b; Coll *et al.*, 1994). The assumption that resistance genotypes are at Hardy-Weinberg equilibrium within sample units has rarely been tested in field populations. In two exceptional cases, Whitehead *et al.*'s (1985) study of diazinon resistance in houseflies and McKenzie's (1984) study of diazinon and dieldrin resistance in the Australian sheep blowfly, no significant deviation from Hardy-Weinberg equilibrium was observed.

High inbreeding is expected to have a significant impact on resistance development whenever the allele is less than fully dominant under field treatment conditions. Inbreeding causes a significant proportion of resistance alleles in females to be expressed in homozygous individuals, even when the initial prevalence of a resistance allele introduced through migration or mutation may be very low. 'Functional' haploidy in males also leads to rapid evolution, since the males' maternally derived genotype is directly subject to

selection. Both inbreeding and 'functional' haplodiploidy undermine any treatment strategy which relies on high mortality of heterozygotes and moderate immigration of susceptibles to thwart resistance development in its early stages (Roush & Daly, 1990; Tabashnik, 1990).

References

Bergamin, J. (1943) Contribução para o conhecimento da biologia da broca do café *Hypothenemus hampei* (Ferrari, 1867) (Col. Ipidae). *Arquivos do Instituto Biologico (São Paulo)* 14, 31–72.

Borsa, P. & Gingerich, D.P. (1995) Allozyme variation and an estimate of the inbreeding coefficient in the coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae). *Bulletin of Entomological Research* 85, 21–28.

Borsa, P. & Kjellberg, F. (1996) Experimental evidence for pseudo-arrhenotoky in the scolytid beetle, *Hypothenemus hampei* (Coleoptera: Scoytidae). *Heredity* 76, 130–135.

Brun, L.O. & Suckling, D.M. (1992) Field selection for endosulfan resistance in coffee berry borer (Coleoptera: Scolytidae) in New Caledonia. *Journal of Economic Entomology* 85, 325–334.

Brun, L.O., Marcillaud, C., Gaudichon, V. & Suckling, D.M. (1989) Endosulfan resistance in *Hypothenemus hampei* (Coleoptera: Scolytidae) in New Caledonia. *Journal of Economic Entomology* 82, 1311–1316.

Brun, L.O., Marcillaud, C., Gaudichon, V. & Suckling, D.M. (1994) Cross resistance between insecticides in coffee berry borer, *Hypothenemus hampei* (Coleoptera: Scolytidae) from New Caledonia. *Bulletin of Entomological Research* 84, 175–178.

Brun, L.O., Stuart, J., Gaudichon, V., Aronstein, K. & ffrench-Constant, R.H. (1995a) Functional haplodiploidy: A mechanism for the spread of insecticide resistance in an important international insect pest. *Proceedings of the National Academy of Sciences, USA* 92, 9861–9865.

Brun, L.O., Suckling, D.M., Roush, R.T., Gaudichon, V., Preisler, H. & Robertson, J.L. (1995b) Genetics of endosulfan resistance in *Hypothenemus hampei* (Coleoptera: Scolytidae): implications for mode of sex determination. *Journal of Economic Entomology* 88, 470–474.

Caprio, M.A. & Tabashnik, B.E. (1992a) Allozymes used to estimate gene flow among populations of diamondback moth (Lepidoptera: Plutellidae) in Hawaii. *Environmental Entomology* 21, 808–816.

Caprio, M.A. & Tabashnik, B.E. (1992b) Gene flow accelerates local adaptation among finite populations: Simulating the evolution of insecticide resistance. *Journal of Economic Entomology* 85, 611–620.

Coll, M., de Mendoza, L.G. & Roderick, G.K. (1994) Population structure of a predatory beetle: the importance of gene flow for intertrophic level interactions. *Heredity* 72, 228–236.

Corbett, G.H. (1933) Some preliminary observation on the coffee berry beetle borer, *Stephanoderes (Cryphalus) hampei* Ferr. *Malayan Agricultural Journal* 21, 8–22.

Crouau-Roy, B. (1988) Genetic structure of cave-dwelling beetle populations: significant deficiencies of heterozygotes. *Heredity* 60, 321–327.

Falconer, D.S. (1989) *Introduction to quantitative genetics*. 3rd edn. 438 pp. Harlow, Essex, Longman Scientific and Technical.

ffrench-Constant, R.H., Steichen, J.C. & Brun, L.O. (1994) A molecular diagnostic for endosulfan insecticide resistance in the coffee berry borer *Hypothenemus hampei* (Coleoptera: Scolytidae). *Bulletin of Entomological Research* 84, 11–16.

Gingerich, D.P. (1996) *Dynamics of insecticide resistance evolution in* Hypothenemus hampei *(Coleoptera, Scolytidae): Treatment regime and population structure*. M.S. thesis, Cornell University.

Giordanengo, P. (1992) *Biologie, éco-éthologie et dynamique des populations du scolyte des grains de café*, Hypothenemus hampei Ferr. *(Coleoptera, Scolytidae), en Nouvelle-Calédonie*. 109 pp. Doctoral thesis, Université de Rennes I.

Grasela, J.J. & Steiner, W.W.M. (1993) Population genetic structure among populations of three predaceous nabid species: *Nabis alternatus* Parshley, *Nabis roseipennis* Reuter and *Nabis americoferous* Carayon (Hemiptera: Nabidae). *Biochemical Systematics and Ecology* 21, 813–823.

Hamilton, W.D. (1967) Extraordinary sex ratios. *Science* 156, 477–488.

Hargreaves, H. (1926) Notes on the coffee berry borer (*Stephanoderis hampei*, Ferr.) in Uganda. *Bulletin of Entomological Research* 16, 347–354.

Hartl, D.L. & Clark, A.G. (1989) *Principles of population genetics*. 2nd edn. 682 pp. Sunderland, Massachusetts, Sinauer Associates.

King, P.S. (1988) Distribution and genetic structure of two allopatric beetle (Coleoptera: Melyridae) species on rock outcrops in the Southeast. *Annals of the Entomological Society of America* 81, 890–898.

Kirkendall, L.R. (1993) Ecology and evolution of biased sex ratios in bark and ambrosia beetles. pp. 235–345 *in* Wrensch, D.L. & Ebbert, M.A. (Eds) *Evolution and Diversity of Sex Ratio in Insects and Mites*. New York, Chapman & Hall.

Le Pelley, R.H. (1968) *Pests of coffee*. 590 pp. London, Longmans, Green and Co.

López, A. & Frérot, B. (1993) Statut physiologique des femelles colonisatrices de *Hypothenemus hampei* (Ferr.). Vol. 2 pp. 831–832 in *15ème Colloque Scientifique International sur le Café*, 6–11 June 1993, Montpellier, France. Association Scientifique Internationale du Café.

Mathieu, F., Brun, L.O. & Frérot, B. (1993) Dynamique de sortie de *Hypothenemus hampei* en présence de cerises vertes. Vol. 2 pp. 833–836 in *15ème Collogue Scientifique International sur le Café*, 6–11 June 1993, Montpellier, France. Association Scientifique Internationale du Café.

McKenzie, J.A. (1984) Dieldrin and diazinon resistance in populations of the Australian sheep blowfly, *Lucilia cuprina*, from sheep-grazing areas and rubbish tips. *Australian Journal of Biological Science* 37, 367–374.

Muñoz, R. (1989) Ciclo biológico y reproducción partenogenética de la broca del fruto del cafeto *Hypothenemus hampei* (Ferr.). *Turrialba* 39, 415–421.

Nei, M. (1977) F-statistics and analysis of gene diversity in subdivided populations. *Annals of Human Genetics* 41, 225–233.

Parkin, C.S., Brun, L.O. & Suckling, D.M. (1992) Spray deposition in relation to endosulfan resistance in coffee berry borer (*Hypothenemus hampei*) (Coleoptera: Scolytidae) in New Caledonia. *Crop Protection* **11**, 213–220.

Prout, T. & Barker, J.S.F. (1993) F-statistics in *Drosophila buzzatii*: selection, population size and inbreeding. *Genetics* **134**, 369–375.

Reilly, L.M. (1987) Measurements of inbreeding and average relatedness in a termite population. *American Naturalist* **130**, 339–349.

Roush, R.T. & Daly, J.C. (1990) The role of population genetics in resistance research and management. pp. 97–152 *in* Roush, R.T. & Tabashnik, B.E. (*Eds*) *Pesticide resistance in arthropods*. New York, Chapman and Hall.

Rowell-Rahier, M. (1992) Genetic structure of leaf-beetle populations: Microgeographic and sexual differentiation in *Oreina cacaliae* and *O. speciosissima*. *Entomologia Experimentalis et Applicata* **65**, 247–257.

Tabashnik, B.E. (1990) Modeling and evaluation of resistance management tactics. pp. 153–182 *in* Roush, R.T. & Tabashnik, B.E. (*Eds*) *Pesticide resistance in arthropods*. New York, Chapman and Hall.

Ticheler, J.H.G. (1961) Étude analytique de l'épidémiologie du scolyte des graines de café *Stephanoderes hampei* Ferr., en Côte d'Ivoire. *Mededelingen Landbouwhogeschool te Wageningen* **61**, 1–49.

Weir, B.S. & Cockerham, C.C. (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

Whitehead, J.R., Roush, R.T. & Norment, B.R. (1985) Resistance stability and coadaptation in diazinon-resistant house flies (Diptera: Muscidae). *Journal of Economic Entomology* **78**, 25–29.

Wright, S. (1969) *Evolution and the genetics of populations. Vol. II. The theory of gene frequencies*. 511 pp. Chicago, University of Chicago Press.