Genetic estimates of dispersal ability in the leucaena psyllid predator Curinus coeruleus (Coleoptera: Coccinellidae): implications for biological control

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Abstract
The leucaena psyllid, Heteropsylla cubana Crawford, can devastate plantings of Leucaena leucocephala (Leguminosae), an economically important tree species in the Pacific Islands, Southeast Asia, Australia, India, Africa, and elsewhere. The predatory beetle, Curinus coeruleus Mulsant (Coleoptera: Coccinellidae), has been introduced into many of these areas from Hawaii for biological control of the psyllid. In this study, collections of C. coeruleus were made from 11 populations on four islands in the Hawaiian archipelago to determine population structure and estimate levels of gene flow. Over all populations, a measure of population subdivision, \( \theta \), was 0.095, and the estimate of \( N_e/m \), the average migration rate, was 2.46. Values for the individual islands were 0.02, 0.12, 0.24 and 0.05 for Kauai, Hawaii, Maui, and Oahu, respectively. Estimated levels of gene flow between populations were not correlated with geographic distance, therefore isolation by distance does not appear to be an important process structuring C. coeruleus populations. Gene flow estimates can be used to characterize dispersal capabilities in insects or other organisms released for biological control. In this case, the inferior dispersal ability of C. coeruleus likely limits its rapid widespread establishment during release programmes.

Introduction
Curinus coeruleus Mulsant (Coleoptera: Coccinellidae) was introduced into Hawaii from Mexico in 1922 for biological control of the coconut mealybug, Nipaecoccus nipae (Maskell) (Homoptera: Pseudococcidae) (Swezey, 1923; Funasaki et al., 1988). It became established and persisted in Hawaii, occasionally found in association with N. nipae, Chrysomphalus aonidum (Linnaeus) (Diaspididae), Aleurodicus dispersus Russell (Aleyrodidae) and Aphis nerii Boyer de Fonscolombe (Aphididae), but was uncommon and never considered an important predator of these pests. Densities of C. coeruleus remained low until the recent accidental introduction to the Hawaiian Islands of the leucaena psyllid, Heteropsylla cubana Crawford (Homoptera: Psyllidae) (Beardsley et al., 1989).
In Hawaii, *Leucaena* is a rapidly growing leguminous tree found in dry to mesic habitats on all islands at elevations up to 700 m. Approximately 60 years ago, it was deliberately broadcast over lowland habitats as a fodder plant (Smith, 1985), and it is now the dominant plant species in many lowland areas. Although in Hawaii it is considered an undesirable weed, in many parts of the world *L. leucocephala* is highly valued for its many uses (shade tree in vanilla, cocoa, tea, and coffee plantations; reforestation and soil reclamation; forage for cattle; fuelwood; timber for light construction; and food for humans) (Beardsley et al., 1989). Feeding by *H. cubana* can devastate the tree and cause economic losses, and, therefore, *C. coeruleus* from Hawaii have been introduced into many countries for biological control of this psyllid. After only a few years, *C. coeruleus* has become a key component of biocontrol programmes in several areas and shows promise in many others (Wagiman et al., 1989; Showler, 1995).

One of the desirable attributes of a biological control agent is a high capacity for dispersal. Ideally, a predator would spread under its own power over the entire range of its host after only one or a few localized introductions (Coppel & Mertins, 1977). The dispersal behaviour of *C. coeruleus* has not been carefully studied. The beetle is common throughout the major Hawaiian Islands wherever *Leucaena* is found, a distribution that suggests *C. coeruleus* has a high dispersal ability like many other entomophagous coccinellids (Hagen, 1962; Ewert & Chiang, 1966; Krasfur et al., 1992; Coll et al., 1994). However, recent observations suggest dispersal by *C. coeruleus* might be limited. Wagiman et al. (1989) observed that beetles had moved only 5 km three years after its introduction and population increase c a coffee plantation in central Java; likewise, in tea plantations in West Java, *C. coeruleus* reproduced well but moved little (Soehardjan, 1989).

A straightforward means to estimate the dispersal capacity of this beetle is to monitor spread of the predators in areas where it has recently been released for biological control. In Hawaii, where *C. coeruleus* is already established throughout the islands, the best means to determine dispersal rates directly is by recapture of marked beetles. Drawbacks to this direct approach are that: (i) it is usually not feasible to conduct mark-recapture studies for more than one or few generations, so rare but significant dispersal episodes may be missed; and (ii) the long distance dispersers may be underestimated because of the limited area over which mark-recapture study can be conducted (Slatkin, 1987, 1996). An alternative method that does not suffer these limitations is to infer levels of movement indirectly from the geographic structure of genetic variation (Slatkin, 1987). In this study, we examined genetic similarity among *C. coeruleus* populations from the Hawaiian archipelago. We tested whether beetles within and between islands are genetically similar, which would be evidence for high dispersal and gene flow, or whether significant population subdivision exists, suggesting limited dispersal.

**Materials and methods**

We collected adult *Curinus coeruleus* from 11 different localities on the islands of Maui (3), Oahu (3) and Hawaii (3) between July–14 June 1993, and on the island of Kauai (2) o
At each site, beetles were sampled from as many different enzymes and eight buffer systems revealed five polymorphic loci in 40 (1/2) of distilled water (in 1.5 ml microcentrifuge electrophoresis, whole beetles were homogenized individually of collecting siblings. Once back in the laboratory, live Leucaena beetles were frozen at −75°C until preparation for electrophoresis.

Cellulose-acetate gels (Helena Laboratories Inc., Beaumont, Texas) were used for allozyme electrophoresis (Richardson et al., 1986; Herbert & Beaton, 1989). Before electrophoresis, whole beetles were homogenized individually in 40 μl of distilled water (in 1.5 ml microcentrifuge tubes) and centrifuged at 6000 rpm for 5 min at 4°C. Gels were run in Zip-zone electrophoresis chambers (Helena Laboratories) maintained at 4°C. A preliminary survey of 20 enzymes and eight buffer systems revealed five polymorphic loci that could be clearly resolved: aldehyde oxidase (AO); E.C. 1.2.3.1); isocitrate dehydrogenase (IDH; two loci: fast [f], slow [s]; E.C. 1.1.1.42); 6-phosphogluconate dehydrogenase (6PGDH; E.C. 1.1.1.44); and phosphoglucomutase (PGM; E.C. 2.7.5.1). A single buffer system, Tris-citrate (0.1 M, pH 8.2) (Richardson et al., 1986), resolved all five loci well. Electrophoretic gels were run at 175 v for either 45 min (AO, IDH, 6PGDH) or 2 h (PGM). Staining techniques were unmodified from Herbert & Beaton (1989). Genotypes were determined for 36 adult beetles from each collection site, except Pohilale (PH; island of Kauai) where 24 beetles were used.

Wright (1951) introduced Fst, a measure of population subdivision for a two-allele one locus system. Fst ranges from 0, indicating no genetic differentiation, to 1, showing complete differentiation. One formulation of Fst is \( Fst = \left( 1 - \frac{Hs}{Ht} \right) \), where Hs is the average heterozygosity within subpopulations and Ht is the predicted total heterozygosity. Similar statistics, such as Nei’s Gst, can be estimated for many alleles at many loci (Nei, 1973, 1977; Birky et al., 1989; Chakraborty & Leimar, 1987). A related measure of population subdivision is \( \theta \), which equals the between-population component of variance (Weir & Cockerham, 1984; Weir, 1990). Fst, Gst and \( \theta \) all estimate the same population parameter. Wright (1951) showed that for neutral alleles, \( Fst = 1/(1 + 4Nm) \), where Ns is the effective population size and Nm is the average rate of migration in an island model of population structure. Rearranging the equation gives \( Nm = 1/(4Fst - 1) \), which is an estimate of gene flow, the average number of migrants between populations each generation (Slatkin, 1987). Especially for moderate \( (Nm=5) \) and high \( (Nm=10) \) levels of gene flow, \( \theta \) is more accurate in its estimate of \( Nm \) than Gst (Slatkin, 1994).

Gst and \( \theta \) were calculated using electrophoretic data for individuals from the 11 different collection sites (unpublished computer program adapted from Weir (1990)), Biosys-1 (Swofford & Saelander, 1981) was used to analyse gene frequency data. \( \chi^2 \) was used to test for deviation from Hardy-Weinberg proportions at each collection site and to test for differences in allele frequencies among populations, within populations, and overall.

Stepping stone, lattice, and island are all models that describe the pattern of movement between subpopulations (Slatkin, 1985, 1987). The stepping stone model assumes migrants move to the nearest neighbouring populations at a higher rate than to more distant subpopulations. The lattice model is a two-dimensional version of the stepping-stone model. Wright’s island model assumes that a migrant from one population is equally likely to move to any of the other populations. To test for isolation by distance, we estimated gene flow (using \( Nm \)) for all pairs of populations and regressed it with straight line geographic distance (log-plots, Slatkin, 1994). To test the significance of the relationship we used the Mantel test (Mantel, 1967; Crowley, 1992) to correct for lack of independency between data points (GKR, unpublished computer program). A significant negative slope is evidence for isolation by distance which supports the stepping stone or lattice models. Also, because Leucaena is distributed primarily around the island edges, circumferential distances between populations within islands might be a better description of dispersal routes than straight line distances; circumferential distances were regressed against \( Nm \) to test for isolation by distance, as well.

Results

Each of the five loci tested exhibited only two electrophorophs; allele frequencies for each population are given in table 1. In our study, allozyme frequencies for each population generally did not deviate significantly from Hardy-Weinberg expectations. Of the 53 possible chi-square tests for Hardy-Weinberg frequencies (11 populations×5 loci), 45 tests were performed, the other ten being omitted because a locus for a particular population was monomorphic (table 1). When the p-value criteria were adjusted for multiple contrasts (by lowering \( \alpha = 1 - [1 - \alpha]^{1/6} \), where \( \alpha = 0.05 \), \( k = 55 \); Sokal & Rohlf, 1981) none of the tests allowed rejection of the null hypothesis that allele frequencies were those expected by Hardy-Weinberg.

The overall estimates of Gst and \( \theta \) in our study were 0.098 and 0.095, respectively (table 2). Bootstrapping over loci, the 95% confidence intervals around \( \theta \) were 0.037–0.15, indicating significant population structure \( (\theta > 0) \). Negative \( \theta \)'s for three loci indicate values that can not be considered different from 0 \( (\theta \text{ is not defined for negative values; Weir, 1990}) \). Estimates of \( \theta \) for the individual islands were 0.017 (95% conf. limits 0.005–0.031) for Kauai, 0.122 (95% conf. limits 0.055–0.187) for Hawaii, 0.238 (95% conf. limits 0.05–0.35) for Maui, and 0.05 (95% conf. limits 0.00–0.104) for Oahu. The relatively high estimates for Gst and \( \theta \) indicate that there was significant genetic differentiation among populations both within islands and over all islands (table 2), which suggests limited gene flow among populations at both geographic scales.

\( \chi^2 \) analysis of gene frequencies confirmed that significant population structure exists between populations within islands. For the islands of Hawaii, Maui, and Oahu, three or four of the five loci showed significantly different allele frequencies for the three populations within the island (table 2). Over all islands, \( \chi^2 \) analysis of gene frequencies showed highly significant differences for all loci, and the estimate of gene flow, \( Nm \), derived from \( \theta \), was 2.4 migrants per generation (95% confidence limits 1.4–6.6) (table 2). There was no significant relationship between \( Nm \) and the straight-line geographic distance between populations (Mantel statistic = −0.006, 95% confidence limits = −0.27–0.25 (fig. 2). There was also no significant relationship between \( Nm \) and circumferential distances, where distances between
populations within islands were measured around the island perimeter (Mantel statistic \( -0.02 \), 95% confidence limits \(-0.29-0.30\)).

**Discussion**

**Barriers to gene flow**

Our evidence for moderate levels of genetic differentiation among populations on each island coupled with the widespread abundance of food on the islands where collections were made, indicates that *C. coeruleus* does not disperse readily. Many possible barriers to gene flow exist. In species with low mobility, gene flow may be restricted by physical barriers such as bodies of water, mountain ranges, or areas without suitable host plants (Roderick, 1996). Ocean is an obvious barrier between the islands in the Hawaiian archipelago and the mountainous relief and deeply carved valleys typical of the volcanic islands form potential barriers to direct gene flow among populations within each island (Simon et al., 1984). A number of ecological factors also can influence the success of migrants in a new habitat, such as asynchrony of migration events with food availability or the reproductive season of the new habitat, assortative mating for resident genotypes, and territoriality in residents (Roderick, 1992). Although *Leucaena* and the psyllid are now abundant over much of the dry and mesic lowland areas ringing the Hawaiian Islands and host plant/prey are
librium is approximately
\[ \frac{2m}{N_e} \]
individuals founding the populations, and extinction events.

migration rate, and \( N_e \), the effective population size. From

estimates for two other coccinellid species (Krafsur et al., 1992; Steiner & Grasela, 1993; Coll et al., 1994). If we estimate the average local population size, \( N_e \), at 1000 (prior to the introduction of \( H. cubana \); effective population size is probably much higher since 1984), our estimate of \( m \) is 0.0025 and the 1/2 time to genetic equilibrium will be 126 generations. If \( C. coeruleus \) can complete four generations per year in Hawaii as it does elsewhere (Napompeth & Maneeratana, 1989), approximately 280 generations would have elapsed between the time the beetle was first introduced (1922) and the time when we made our collections (1993–94). Given these conditions, our populations would be close to genetic equilibrium, as required by the model. However, whether the 11 populations that were sampled became established and persisted since shortly after the introduction of \( C. coeruleus \) 70 years ago is uncertain. Prior to the introduction of \( H. cubana \), \( C. coeruleus \) is reported to have been established on all the major Hawaiian Islands (Funasaki et al., 1989). Historical records of releases of \( C. coeruleus \) are limited to the initial releases of beetles from Mexico made on the islands of Oahu (1391 beetles) and Maui (50 beetles) in 1922 by the Hawaii State Department of Agriculture, and releases of beetles from Trinidad, West Indies made on Oahu in 1953–54 (316 beetles). More beetles were brought into Hawaii from Guatemala in 1962 but there is no record of a release. There is insufficient information to determine whether \( C. coeruleus \) was carried to other islands after the initial releases. Without this information it is impossible to speculate whether the present distribution of \( C. coeruleus \) in the islands is the result of natural dispersal from the initial release sites or human-facilitated dispersal.

**Population structure in other coccinellids**

Estimates of population subdivision have been made for two other predatory coccinellids: in Coleomegilla maculata DeGeer, \( \theta \) was estimated as 0.0015 (\( N_e m = 166.4 \)) (Coll et al., 1994) and \( F_{ST} \) was estimated as 0.14 (\( N_e m = 1.5 \)) (Steiner & Grasela, 1993); and in Coccinella septempunctata Linnaeus the estimate of \( \theta \) was 0.0148 (\( N_e m = 16.6 \)) (Krafsur et al., 1992). Krafsur et al. (1992) suggested that the low \( \theta \) value for \( C. septempunctata \) populations in North America was a reflection of their recent ancestry rather than extensive gene flow: numerous releases of \( C. septempunctata \) from Europe were made in the US between 1957 and 1978, but the first detection of self-sustaining populations and the rapid spread of its geographic distribution did not occur until the 1980s (the \( C. septempunctata \) populations that were detected at this time may actually have been accidental introductions) (Krafsur et al., 1992). Coleomegilla maculata on the other hand is native to North America and populations are likely to have reached genetic equilibrium. Krafsur et al. (1992) also estimated \( \theta \) for three populations of \( C. septempunctata \) from Europe, within the native range of \( C. septempunctata \), and found levels of gene flow (\( \theta = 0.003; N_e m = 83.1 \)) similar to \( C. maculata \) from Maryland (Coll et al., 1994). The disparity in estimates of \( N_m \) between the two \( C. maculata \) studies (166.4 vs. 1.5) may be a reflection of the scale and design of the two studies: populations collected by Coll et al. (1994) were all from corn fields in Maryland (USA) and the maximum distance between populations was about 120 km, whereas the populations examined by Steiner & Grasela

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**Genetic assumptions**

The use of electrophoretic or other genetic information to determine population structure makes the important assumption that genetic structure is largely the consequence of gene flow among populations (Daly, 1989). For this to be valid several assumptions must be met: (i) gene flow must be random with respect to the genotypes studied; (ii) the rate of gene flow must exceed the effects of selection; (iii) the rate of gene flow must exceed a minimum level to offset the effects of genetic drift; and (iv) the population must be at genetic equilibrium, a balance between the forces of gene flow and genetic drift (Daly, 1989). Assumptions (i) and (ii) are likely to be met for allozymes that are considered to be neutral or under very weak selection. Also, if many loci give the same results it is unlikely that all are responding similarly to the same selective force. For assumption (iii), Wright (1951) showed that even one migrant every other generation is sufficient to prevent substantial genetic differentiation due to genetic drift.

Assumption (iv), that populations are in genetic equilibrium, is the most difficult to satisfy; several factors will influence the rate of approach to equilibrium including migration rate, population size, the genetic make-up of the individuals founding the populations, and extinction events. The time in generations for \( \Delta t \) to go half way to equilibrium is approximately

\[ \frac{1}{2} \left( 1 + \frac{N_e}{2m} \right) \]

(Crow & Aoki, 1964). Solving this equation requires an estimate of \( m \), the migration rate, and \( N_e \), the effective population size. From our own data \( N_m \) for \( C. coeruleus \) is estimated as 2.4 migrants per generation; this level of gene flow is comparable to several other beetle species (Daly, 1989), but lower than estimates for two other coccinellid species (Krafsur et al., 1992; Steiner & Grasela, 1993; Coll et al., 1994).
(1993) were from multiple crop types across a five state region in the midwestern United States, with a latitudinal distance between the furthest populations that was 12 times that of the Maryland study. The level of population subdivision in Hawaiian Curinus coeruleus is comparable to the estimate for C. maculata in the midwestern United States (Steiner & Grasela, 1993); the maximum distance between populations was approximately 500 km for Curinus coeruleus and 1000 km for C. maculata.

A relatively large number of C. coeruleus (n=1400) were initially introduced to the Hawaiian Islands. The pattern of genetic differentiation between populations within islands and over all islands may indicate that different parts of the islands were founded by small numbers of individuals. Since 1984, the leucaena psyllid has provided C. coeruleus with a more abundant source of prey and population sizes are now large. Due to the availability of prey, C. coeruleus probably has expanded its distribution within individual islands. Our data, however, indicates that local populations are still distinct and therefore populations have not mixed extensively.

**Implications for biological control**

Dispersal characteristics of C. coeruleus may limit its rapid widespread establishment during release programmes. A system based on mass rearing was developed in Indonesia to overcome this limitation and should serve as a model for other release programmes for this predator. The emphasis in Indonesia was placed on training personnel from government agencies and private industry; these trainees learned to mass-rear C. coeruleus and then returned to the provinces to train small land holders and crop protection staff in these techniques. Within a year, over 4000 people were trained and they produced a total of 350,000 beetles for local releases (Soehardjan, 1989). In 1989, the predator was being mass produced in 14 provinces and 88 smaller substations throughout the country. In addition, farmers were encouraged to collect C. coeruleus from areas where they were thriving and disseminate them to additional fields, a practice that took hold nationwide (Soehardjan, 1989).

By virtue of its location in the middle of the Pacific, Hawaii has been invaded by many immigrant insect species from east and west; more biological control introductions against immigrant species have been initiated in Hawaii than anywhere else in the world (Funasaki et al., 1988). This reservoir of introduced organisms (predatory insects and mites, parasitic insects and fungi, phytophagous insects, dung beetles, predatory snails and amphibians, etc.) could be used to improve the success of biological introductions elsewhere. Most biological control release are made for the first time without prior knowledge of the dispersal characteristics of the released organism. Natural history information about the organism can reveal limits to the range and distribution of the organism in its native environment, but the actual extent of movement of individuals within the range may be poorly understood. Hawaii provides a unique natural laboratory to examine dispersal. Where accurate records of releases of a biological control organism have been kept, present patterns of distribution in the islands can give clues to the vagility of the organism. If sufficient genetic variability can be detected, allozyme electrophoretic or DNA sequence data can be used to determine population structure and estimate migration rates (Roderick, 1996). This information could be used to develop release strategies for biological control programmes in other areas using the species studied or a related species. In addition, studies of population structure can be used to establish baseline genetic data for evaluating the importance of genetic variation in classical biological control programmes (see Roderick, 1992).

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**References**


