

Inbreeding variability and population structure in the invasive haplodiploid palm-seed borer (*Coccotrypes dactyliperda*)

J. P. HOLZMAN,* A. J. BOHONAK,† L. R. KIRKENDALL,† D. GOTTLIEB,‡§ A. R. HARARI§¶ & S. T. KELLEY*

*Department of Biology, San Diego State University, San Diego, CA, USA

†Department of Zoology, University of Bergen, Bergen, Norway

‡Department of Desert Ecology, Blaustein Institute for Desert Research, Ben-Gurion University of the Negev, Be'er-sheva, Israel

§Department of Life Sciences, Ben-Gurion University of the Negev, Be'er-sheva, Israel

¶Department of Entomology, Agricultural Research Organization, The Volcani Center, Bet-Dagan, Israel

Keywords:

bark beetle;
dispersal;
 F_{IS} ;
invasive species;
microsatellites;
population genetics.

Abstract

We investigated the mating system and population genetic structure of the invasive haplodiploid palm-seed borer *Coccotrypes dactyliperda* in California. We focused on whether these primarily inbreeding beetles have a 'mixed-breeding' system that includes occasional outbreeding, and whether local inbreeding coefficients (F_{IS}) varied with dominant environmental factors. We also analysed the genetic structure of *C. dactyliperda* populations across local and regional scales. Based on the analysis of genetic variation at seven microsatellite loci in 1034 individual beetles from 59 populations, we found both high rates of inbreeding and plentiful evidence of mixed-breeding. F_{IS} ranged from -0.56 to 0.90 , the highest variability reported within any animal species. There was a negative correlation between F_{IS} and latitude, suggesting that some latitude-associated factor affecting mating decisions influenced inbreeding rates. Multiple regressions suggested that precipitation, but not temperature, may be an important correlate. Finally, we found highly significant genetic differentiation among sites, even over short geographic distances (< 1000 m).

Introduction

Invasive plant and animal species have altered the composition of countless ecosystems worldwide. Although individual pioneers generally have low probabilities of establishing viable populations (Mack *et al.*, 2000), humans have radically increased the movement of non-native plant and animal species into new environments. This activity has greatly expanded the number of invasive colonization opportunities and has contributed to the successful establishment of many non-native species (Cohen & Carlton, 1998; Nico & Fuller, 1999; Work *et al.*, 2005). Invasive species affect local ecology through competitive exclusion, niche displacement and

predation, often resulting in the extirpation or extinction of native species (Vitousek *et al.*, 1997; Wilcove *et al.*, 1998; Mooney & Cleland, 2001). Introductions of non-native species have resulted in significant losses of local biological diversity and degraded the function of ecosystems (Williamson, 1998). Studies of invasive species population genetic structure can, therefore, provide important information regarding the frequency of introductions, the source populations, and patterns of dispersal across landscapes (e.g. Human & Gordon, 1997; Holway, 1999; Wadsworth *et al.*, 2000; Carol, 2002; Potter & Held, 2002; Carruthers, 2003; Mun *et al.*, 2003; Thomson, 2004).

Theoretically, plants and animals that reproduce through self-fertilization, parthenogenesis, or biparental inbreeding should be particularly effective invaders, as only one individual is required for colonization of a new habitat. Coincidentally, inbreeding bark beetles seem able to colonize new habitats more readily than closely

Correspondence: Scott T. Kelley, Department of Biology, San Diego State University, San Diego, CA 92182-4614, USA.
Tel.: +1 619 594 5371; fax: +1 619 594 5676;
e-mail: skelley@sciences.sdsu.edu

related outbreeding species (Jordal *et al.*, 2001). Of the 50 species of exotic scolytids established in the continental United States, 37 are inbreeding (Haack, 2001). The inbreeding palm-seed borer *Coccotrypes dactyliperda* Fabricius (Coleoptera: Scolytinae) provides an especially good example of a successful colonizer. *Coccotrypes dactyliperda* is one of approximately 1400 described species in the Xyleborini tribe of predispersal, sib-mating beetles. This tribe includes many seed-borers, as well as wood-boring ambrosia fungus-feeding beetles (Wood, 1982; Jordal *et al.*, 2002). *Coccotrypes dactyliperda* breeds in the seeds of various palms, especially those found in the genus *Phoenix*. *Coccotrypes dactyliperda* is haplodiploid and can be further classified as 'Arrhenotokous': dwarfed haploid males develop from unfertilized eggs and mate with their diploid sisters prior to dispersal (predispersal mating; Herfs, 1950). However, multiple females regularly enter and breed in the same seeds, sometimes even through the same entrance holes (Kirkendall & Gottlieb, unpublished), allowing for the possibility for both inbreeding and outbreeding. Behavioural studies also show that these beetles will outbreed under some conditions, suggesting that they may have a 'mixed mating' system (D. Gottlieb *et al.*, unpublished data).

Kirkendall (1993) outlined three main factors favouring the evolution of sib-mating in tropical bark beetles: (i) breeding in spatially limited resources, such as seeds, fruits or the pith of twigs; (ii) cave-type gallery systems (nests) in which eggs are laid in clusters and larvae develop in close proximity; and (iii) year-round continuous breeding opportunities in the tropics. All populations of *C. dactyliperda* conform to the first two factors (i.e. seed-breeding and cave-type gallery systems). However, continuous breeding opportunities may not exist for all populations, particularly for those in more temperate latitudes. Two factors that are likely to affect breeding opportunities are climate (e.g. temperature) and seed production. Climatic conditions are more highly variable in temperate latitudes, and many plant species have greater variability in seed production at higher latitudes (Koenig & Knops, 2000; Kelly & Sork, 2002). Therefore, if continuous breeding opportunities favour inbreeding and discontinuity favours outbreeding, one would expect a greater degree of outbreeding in populations that are found in higher latitudes. Interestingly, inbreeding bark beetles show the opposite latitudinal trend of parthenogenetic plants and animals. Although parthenogenesis is more common at high latitudes and elevations (Glesener & Tilman, 1978; Bell, 1982; Bierzychudek, 1987), inbreeding is *less common* and most inbreeding bark beetles are found in the tropics (Kirkendall, 1993). To explain this contrary latitudinal trend, Kirkendall (1993) suggested that tradeoffs between early sexual maturation, successful over-wintering and dispersal do not favour the evolution of predispersal mating in temperate climates. Thus, we expected lower rates of predispersal mating (biparental inbreeding) as *C. dactyliperda* expands

its ranges into the mid-latitudes. The relative recent invasion of this beetle along the California coast allowed a unique opportunity to test the latitudinal gradient hypothesis.

Coccotrypes dactyliperda was first collected in the USA in 1915 (Atkinson & Peck, 1994), and has been established in California for more than 80 years. In North America, *C. dactyliperda* has also been collected in Arizona, Florida, Texas, and Baja California, Mexico (Wood, 1982). This species is an excellent colonist because only one un-inseminated female is theoretically required to start a population (Kirkendall, 1993), and females are known to live long enough to mate with their haploid sons (Herfs, 1950; Kirkendall & Gottlieb, personal observation). Haplodiploid species may also have an advantage over sexually reproducing diploids when immigrating to new areas, as the effects of inbreeding depression are mitigated by the purging of deleterious alleles through direct exposure to selection (Werren, 1993; Peer & Taborsky, 2005; but see Darvill *et al.*, 2006).

Confusingly, the term 'inbreeding' has been broadly used to describe a variety of different individual- and population-level phenomena. In some cases, 'inbreeding' is simply equated with mating with close relatives. However, in population genetics, inbreeding is defined as mating with relatives more often than would occur in a reference population with random mating (concepts reviewed in Keller & Waller, 2002). The local inbreeding coefficient, F_{IS} , uses the local gene pool as the point of reference. By definition, organisms that randomly mate within a single gene pool choose mates without regard to spatial proximity. If the gene pool is of finite size and mating is truly random, relatives will occasionally mate due to chance encounters. As a result, local inbreeding ($F_{IS} > 0$) is typically attributed to a *behavioural choice* to mate preferentially with relatives. In contrast, the overall inbreeding coefficient F_{IT} uses genetic data from across the entire species as a reference, combining the effects of (i) local nonrandom mate choice (F_{IS}) with (ii) divergence among gene pools (F_{ST}). If the species contains a sufficiently large number of populations, the reference approximates an infinitely large Hardy-Weinberg population model. The distinction is important for empirical data interpretation because nonrandom mate choice will affect both F_{IS} and F_{IT} , but a bottleneck in population size will not affect F_{IS} unless the local probability of mating with relatives is density dependent.

In this study, we investigated microsatellite variation within and among populations of *C. dactyliperda* in California, USA. Our first goal was to quantify variability in the local inbreeding coefficient F_{IS} among populations of *C. dactyliperda*, and test whether F_{IS} correlates with latitude. Substantial variation in inbreeding among populations would indicate the existence of a mixed-mating system and suggest that these beetles choose to outbreed (or have evolved different breeding strategies) in specific

environmental conditions. Assuming sufficient statistical power, very weak or no correlation between F_{IS} and latitude may indicate that environmental variability exerts an inconsistent influence on mating system, or that too little time has elapsed for patterns to be established. In contrast, a significant negative correlation would support the hypothesis that outbreeding (i.e. lower values of F_{IS}) is more advantageous at higher latitudes, which have more seasonal environments (e.g. Mitton, 1997). A significant positive relationship between F_{IS} and latitude (e.g. Krakowski *et al.*, 2003) would be more difficult to explain in terms of adaptive or nonadaptive mate choice. For example, if gene pool sizes decline as a function of latitude, individuals at the northern end of a species' range will obviously mate more often with relatives than those at the southern end (increasing F_{IT} but not necessarily F_{IS}). A positive correlation between F_{IS} and latitude would probably represent other phenomena, such as selection for increased heterozygosity at lower latitudes. In addition to exploring patterns of inbreeding, we also analysed population structure in this invasive species across spatial scales that ranged from local to regional. For the local scale, we focused on two urban neighbourhoods in southern California where the earliest invasion dates were 1914 and 1930, respectively. Finally, we compare our results with those of a parallel study on the same species carried out in Israel.

Materials and methods

Sample collection

Female *C. dactyliperda* was collected from Canary Island Date Palm seeds (*Phoenix canariensis*) between March 2004 and November 2005. Sampling during 2004 focused on quantifying genetic divergence at the largest spatial scale, with 726 individuals collected from seeds under 40 palms across California. We collected only diploid females and only one per seed. We operationally defined a population of beetles as individuals collected within a 5-m radius of a single host palm, with only one palm sampled per population. We made the assumption that beetles under a single tree follow the evolutionary (or population genetic) definition of populations, in which all individuals can potentially mate without regards to spatial proximity. In most cases, population discrimination proved uncomplicated, because host trees were separated by large areas of unsuitable urban habitat. These 40 populations were grouped into 13 cities (major metropolitan areas) based on geographic distance and political boundaries: San Diego (SD), Oceanside (OS), El Centro (EC), Avalon on Santa Catalina Island (AV), San Onofre (SO), Laguna (LG), Los Angeles (LA), Oxnard (OX), Ventura (VE), Santa Barbara (SB), Santa Maria (SM), San Los Obispo (SLO) and San Francisco (SF) (Fig. 1; Table 1).

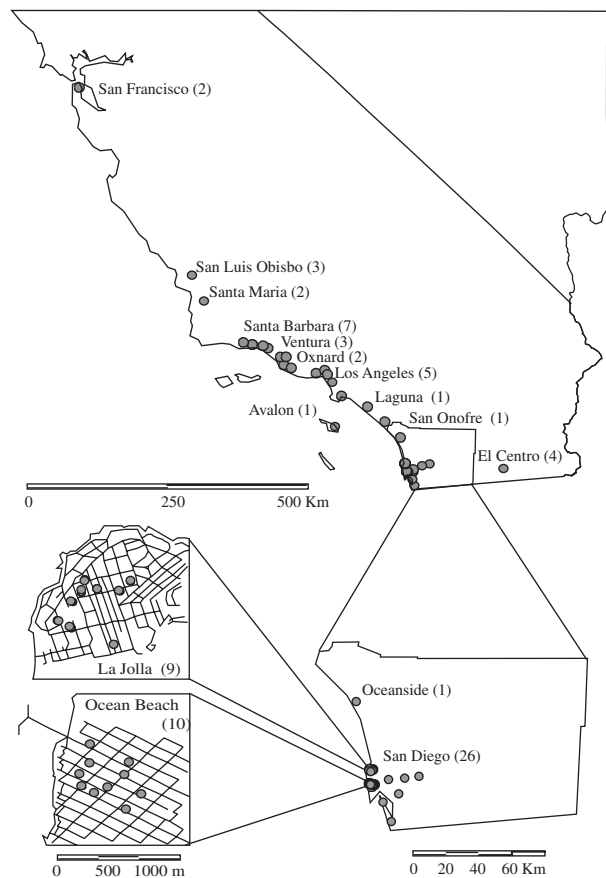


Fig. 1 Distribution of sampled *Coccotrypes dactyliperda* populations throughout California at state-wide, county and neighbourhood levels.

We collected 308 individuals from 19 additional populations during 2005 to quantify local population structure. The small-scale sampling focused on the communities of Ocean Beach (OB) and La Jolla (LJ; near San Diego, CA), adhering as closely as possible to regularly spaced grids (modelled after Mardulyn & Milinkovitch, 2005).

DNA extraction, PCR, allelic scoring

Genomic DNA was extracted from individual beetles, which were crushed in 1.5-mL Eppendorf tubes using a plastic insect pestle. Tissue was incubated in 3 μ L of proteinase K (10 mg mL⁻¹) and 600 μ L of homogenization buffer [5 mL of 2 M Tris-HCl pH 9.1, 0.58 g NaCl, 6.85 g Sucrose, 10 mL EDTA (500 mM), 5 mL 10% SDS in 100 mL of H₂O], and incubated overnight at 55 °C. After adding 50 μ L of 8 M KoAc, the sample was vortexed and placed on ice for 15 min. Samples were then centrifuged at 12 879 *g* at 4 °C for 10 min. The supernatant was removed, transferred to a clean Eppendorf tube, and washed with 600 μ L 1 : 1 phenochloroform : isoamyl alcohol. The mixture was then centrifuged at

Table 1 Sampling sites for *Cocotrypes dactyliperda* in California, USA.

City	Code	Latitude (N)	Longitude (W)	Elevation (m)
San Diego	SD1	32.578717	-117.128400	2
	SD2	32.699767	-117.176567	2
	SD3	32.726050	-117.231267	15
	SD4	32.734417	-117.147533	98
	SD5	32.762917	-117.145367	104
	SD6	32.774733	-117.073083	127
	SD7	32.790967	-116.961700	136
Ocean Beach	OB1	32.741867	-117.250700	8
	OB2	32.743283	-117.253067	10
	OB3	32.744483	-117.252167	0
	OB4	32.743450	-117.249050	10
	OB5	32.741133	-117.247583	12
	OB6	32.746533	-117.251950	20
	OB7	32.744483	-117.248617	7
	OB8	32.741383	-117.251767	4
	OB9	32.741883	-117.252583	5
	OB10	32.739467	-117.248950	34
La Jolla	LJ1	32.843483	-117.276767	32
	LJ2	32.842500	-117.277633	29
	LJ3	32.839733	-117.277767	20
	LJ4	32.840567	-117.279233	20
	LJ5	32.844883	-117.276417	20
	LJ6	32.843917	-117.275217	20
	LJ7	32.838050	-117.273467	20
	LJ8	32.843633	-117.272817	30
	LJ9	32.844667	-117.271750	34
Oceanside	OS1	33.189667	-117.347733	12
El Centro	EC1	32.776983	-115.560167	0
	EC2	32.778400	-115.568550	0
	EC3	32.780433	-115.583867	3
	EC4	32.784950	-115.560967	0
San Onofre	SO1	33.596683	-117.869367	18
Avalon	AV1	33.340500	-118.327783	22
Laguna	LG1	33.398450	-117.594417	29
Los Angeles	LA1	33.735750	-118.279517	16
	LA2	33.879317	-118.401867	10
	LA3	34.019700	-118.503633	88
	LA4	34.034983	-118.680917	8
	LA5	34.074083	-118.546767	386
Oxnard	OX1	34.146300	-119.191983	3
	OX2	34.196250	-119.184983	1
Ventura	VE1	34.266450	-119.270933	2
	VE2	34.279767	-119.278583	58
	VE3	34.280117	-119.248367	69
Santa Barbara	SB1	34.364283	-119.446317	19
	SB2	34.393467	-119.518617	13
	SB3	34.397733	-119.713483	2
	SB4	34.410517	-119.690567	2
	SB5	34.418700	-119.704300	12
	SB6	34.420683	-119.656417	14
	SB7	34.436500	-119.830783	11
Santa Maria	SM1	34.954317	-120.426967	67
	SM2	34.955200	-120.441967	60
	SM3	34.956067	-120.431700	68
San Luis Obispo	SLO1	35.281350	-120.653633	88
	SLO2	35.290617	-120.649650	98
	SLO3	35.295717	-120.663133	90
San Francisco	SF1	37.757317	-122.424483	46
	SF2	37.765317	-122.431983	18

8 944 *g* for 5 min and the supernatant (~600 μL) was removed and transferred to a clean Eppendorf tube. A final 600 μL chloroform extraction was performed to remove trace phenol and samples were centrifuged at 8 944 *g* for 5 min. The supernatant was then mixed with 1.25 mL of 100% ethanol and incubated at -70°C for 30 min for DNA precipitation. After centrifugation at 12 879 *g* for 15 min, the DNA pellet was dried and resuspended in 75 μL of H_2O . DNA was diluted to a final concentration of 60 $\text{ng } \mu\text{L}^{-1}$, estimated using a ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

Microsatellite primers were selected from among a set previously developed for *C. dactyliperda* and *Cocotrypes carpophagus* (Berg *et al.*, 2003). The following six loci were used on all samples: Cdact1 Cdact2, Cdact6, Cdact10, Cdact13 and Ccarp10. A seventh locus (Cdact5) was added in 2005 for increased resolution of local population structure in OB and LJ. Fluorescent dye-labelled versions of these primers (FAM-6, VIC, PET and NED) were obtained from Applied BioSystems (Foster City, CA, USA). PCR temperatures and cycling parameters followed Berg *et al.* (2003). PCR amplifications were performed in a total volume of 25 μL , containing 10 mM Tris-HCl (pH 8.3), 3 mM MgCl_2 , 0.5 U *Taq* polymerase (Promega, San Luis Obispo, CA, USA), 50 μM of dNTPs, 40–60 $\text{ng } \mu\text{L}^{-1}$ of DNA template, and 5 μM of fluorescent primer. PCR products based on differing dye colours were not combined for genotyping, with dilution amounts estimated after visualization on agarose gels. Products were diluted from 1 : 30 to 1 : 60 depending on band brightness. Three microlitres of diluted PCR products was mixed with 8 μL hi-di formamide and 0.4 μL of GENESCAN-500 LIZ-labelled molecular weight standard (Applied BioSystems). The samples were then denatured at 95°C for 1 min before loading onto an ABI 3100 automated sequencer. ABI default genotyping run conditions for Pop-4 polymer were used (Applied BioSystems), with the run time extended to 1700 s. The GENEMAPPER 3.7 software was used to score alleles (Applied BioSystems).

Intrapopulation and total genetic diversity

We analysed three data sets: (i) the small-scale OB dataset containing 143 individuals from 10 trees (seven loci), (ii) the small-scale LJ dataset containing 143 individuals from nine trees (seven loci), and (iii) the entire dataset containing 1034 individuals from all 59 populations (includes the OB and LJ data sets; six to seven loci per population). The following summary statistics were calculated for each data set: number of alleles per locus, effective number of alleles (n_e), observed heterozygosity (H_O), total and average gene diversity (H_T and H_S ; Nei, 1978), total and average allelic richness (R_T and R_S ; El Mousadik & Petit, 1996), and the inbreeding coefficients F_{IS} and F_{IT} . H_T and H_S are equivalent to expected heterozygosity calculated with a

sample size correction, overall and at the level of the population. R_T and R_S estimate the number of alleles per locus overall and in a population, correcting for different sample sizes in each population. All statistics were computed with `FASTAT` 2.9 (Goudet, 1995). Microsatellite loci were also examined for evidence of linkage disequilibrium using the Fisher method in `GENEPOP` 3.3 (Rousset & Raymond, 1995).

Environmental correlates of inbreeding and genetic diversity

We obtained temperature and precipitation data for each population, averaged over the years 1961–1990, from the Western Regional Climate Center (<http://www.wrcc.dri.edu/>) to estimate the relative contributions of climatic factors and latitudinal gradients to the levels of inbreeding and genetic diversity. Testing these hypotheses through techniques such as multiple regression is challenging, as climatic variables such as minimum and maximum temperature correlate strongly with each other, as well as with covariates such as altitude. We addressed strong collinearity among the potential predictors of inbreeding as follows:

- 1 Across the 59 populations, we used a preliminary principal components analysis (PCA) to reduce dimensionality for nine potential predictors related to temperature. These predictors were minimum and maximum temperatures from January to August (max.–min. temp.) for each month [(max. August temp.–max. January temp.), (min. August temp.–min. January temp.) and (max. August temp.–min. January temp.)].
- 2 The first two principal components explained 94.4% of the total variance in the environmental data. PC1 reflected similar correlations (negative loadings) for all variables except minimum January temperature. PC1 scores were highest in coastal SF (which is generally cool, but has relatively high min. January temperatures), followed by Avalon and the coastal populations in San Diego County. PC1 scores were lowest in the desert populations from EC. PC2 loaded positively on six variables, most notably min. and max. January temperatures, and min. August temperatures. PC2 loaded negatively on temperature ranges for the months of January and August. Overall, PC2 separated the relatively warm southern populations (highest scores in EC, OB, LJ, SD) from cooler, more stable populations (lowest scores in SLO, SM).
- 3 We performed univariate and multiple regressions of four summary genetic statistics (R_S , H_O , H_S and F_{IS}) on latitude, elevation, annual precipitation, temperature PC1 and temperature PC2. PCA and regressions were performed in `DATADESK` 6.2.1 (Velleman, 1997). Population SM2 was excluded from the F_{IS} analysis because only one allele was present there, and F_{IS} could not be calculated.

Population structure

Despite their popularity in population genetic analyses, we did not analyse population structure with individual-based clustering algorithms (e.g. Pritchard *et al.*, 2000), because they rely heavily on the assumption that mating is random within populations or at least constant across populations. Instead, we analysed population structure with a combination of exact tests and F -statistics (see Waples & Gaggiotti, 2006 for a review of statistical power using various methods). Wright's (1951) F_{ST} was calculated between all population pairs within each data set using `ARELQUIN` 3.0 (Excoffier *et al.*, 2005). The statistical significance of each value was calculated from 10 000 permutations, with a significance level of $\alpha = 0.05$ after Bonferroni correction for multiple tests. A hierarchical model of differentiation was also estimated using the program `TFPGA` (Miller, 1998). Divergence among populations within cities was denoted F_{SC} , and divergence among cities was denoted F_{CT} .

We tested for patterns of isolation by distance (IBD) among populations using Isolation by Distance Web Service `IBDWS` 2.0 (Jensen *et al.*, 2005). Rousset's (1997) genetic distance [$F_{ST}/(1 - F_{ST})$] was evaluated against the Euclidean (straight line) geographic distance among population pairs. As with many analyses of IBD (e.g. Slatkin, 1993), log transformation of both variables was used to achieve linearity. Negative values of Rousset's distance were replaced with 0.0001 prior to log transformation. (The next smallest positive value was 0.0006.) Mantel (1967) tests were performed with 10 000 random permutations to test for statistically significant associations between pairwise genetic distance matrix and the geographic distance matrix. IBD analyses were performed on small spatial scales (OB and LJ, separately) and the total data set. We visualized nonlinear aspects of IBD with a LOWESS smoothing function (span = 20%) in `DATADESK` 6.2.1 (Velleman, 1997).

Finally, we visualized the spatial structure of the OB and LJ small-scale data sets using Alleles In Space (`AIS`) (Miller, 2005). This program generates three-dimensional genetic landscape surfaces, where the Z -axis (height) represents genetic distance, and the X and Y -axes represent UTM coordinates. `AIS` analyses were performed using the default genetic distances, which are generated by (i) calculating a simple index of allelic similarity between all individuals, (ii) performing a linear IBD regression of these genetic distances against Euclidean geographic distance for all pairs of individuals, (iii) interpolating a genetic surface using the residuals from that regression and a Delauney triangulation network. Output grid sizes were varied (20×20 , 50×50 and 100×100) along with distance weights ($\alpha = 0.5$, 1.0 and 2.0), to assure that our results were robust to these parameters. Because the genetic landscape surfaces were extremely similar, we report here only grid size 50×50 ,

$\alpha = 1.0$ for the local analyses, and grid size 100×100 , $\alpha = 1.0$ for the statewide analysis.

Results

Intrapopulation and overall genetic diversity

Table 2 summarizes genetic diversity within populations, and the local inbreeding coefficients. The total number of alleles per locus (n_a) ranged from 3 to 17, with a total of 67 alleles scored over the seven loci. Gene diversity (H_S) varied broadly from 0 to 0.362, and was generally higher than observed heterozygosity (H_O). All populations except SM2 contained at least two polymorphic loci.

The local inbreeding coefficient F_{IS} was positive in 81% of the populations surveyed, and significantly greater than zero in 29–55% of the populations (Tables 3 and 4). An average value of $F_{IS} = 0.27$ across populations confirmed a general tendency towards inbreeding in *C. dactyliperda*. However, F_{IS} -values varied considerably from -0.562 to 0.902 among populations, and were negative in 11 cases. Tests for genotypic disequilibrium did not detect significant deviations from the null hypothesis of independent assortment ($P > 0.40$ for all pairwise comparisons among loci).

The locus Ccarp10 had a 25% PCR failure rate, compared with 0–9% at other loci. Because this primer was initially developed for *C. dactyliperda*'s sister species *C. carpophagus* Hornung, these failures may represent null alleles, which could bias our results. However, levels of diversity at Ccarp10 were similar to other loci. More importantly, the presence of null alleles is expected to decrease disproportionately H_O , resulting in an unusually high value for the local inbreeding coefficient F_{IS} . No such effect was apparent (Table 2). Analyses performed without the Ccarp10 locus (not presented) were qualitatively similar to those performed with all loci.

Table 2 Comparison of genetic diversity found at seven microsatellite loci in *Coccytrypes dactyliperda*: average number of alleles (n_a), effective number of alleles (n_e), average observed heterozygosity (H_O), overall gene diversity (H_T), overall allelic richness (R_T), and fixation index between individuals and total data set (F_{IT}).

Locus	n	n_a	n_e	H_O	H_T	R_T	F_{IT}
Cdact1	1013	10	1.5	0.231	0.334	9.53	0.308**
Cdact2	967	10	1.4	0.045	0.273	9.71	0.837**
Cdact5	292	3	1.5	0.192	0.338	2.985	0.433**
Cdact6	932	7	2.4	0.156	0.58	6.766	0.732**
Cdact10	1024	9	1	0.023	0.036	8.537	0.344**
Cdact13	942	11	1.1	0.067	0.079	9.986	0.154**
Ccarp10	770	17	1.5	0.188	0.34	17	0.446**
Mean	849	9.6	1.5	0.130	0.283	9.216	0.465
SD		4.2	0.4	0.08	0.182	4.219	0.241

** $P < 0.001$.

Table 3 Genetic variability of six microsatellites in of *Coccytrypes dactyliperda*: number of individuals genotyped (N), number of polymorphic loci (P), average number of alleles (n_a), average observed heterozygosity (H_O), average expected heterozygosity (H_E), average allelic richness (R_S), and fixation index between individuals and the local population (F_{IS}).

Site	N	P	n_a	H_O	H_E	R_S	F_{IS}
SD1	16	2	1.33	0.01	0.031	1.33	0.667*
SD2	18	3	1.50	0.065	0.102	1.33	0.357
SD3	14	3	1.83	0.060	0.079	1.83	0.221
SD4	18	3	1.67	0.135	0.172	1.67	0.217**
SD5	20	4	1.83	0.129	0.154	1.83	0.164
SD6	16	2	1.33	0.094	0.103	1.33	0.094
SD7	17	3	1.67	0.091	0.131	1.66	0.308*
OS1	19	3	2.00	0.029	0.277	1.97	0.902**
SO1	32	6	2.83	0.129	0.254	2.12	0.506**
EC1	19	5	2.83	0.134	0.298	2.82	0.560**
EC2	13	3	1.83	0.059	0.176	1.63	0.550**
EC3	14	3	1.50	0.075	0.152	1.50	0.614**
EC4	14	4	1.83	0.132	0.174	1.83	0.250**
AV1	19	4	2.50	0.176	0.199	2.48	0.120**
LG1	18	5	2.33	0.258	0.295	2.27	0.128**
LA1	16	4	1.83	0.115	0.107	1.83	-0.071
LA2	12	2	2.00	0.046	0.227	1.98	0.808**
LA3	14	6	3.67	0.077	0.348	2.80	0.788**
LA4	17	4	2.17	0.162	0.196	2.15	0.178
LA5	16	2	1.50	0.115	0.136	1.50	0.162
OX1	19	3	1.50	0.184	0.120	1.50	-0.562**
OX2	19	6	2.17	0.219	0.154	2.17	-0.438**
VE1	15	4	1.67	0.233	0.284	1.67	0.183
VE2	16	3	1.67	0.135	0.133	1.67	-0.018
VE3	19	4	2.00	0.281	0.254	2.00	-0.107**
SB1	16	5	2.00	0.177	0.251	2.00	0.300**
SB2	19	5	2.00	0.097	0.159	1.98	0.400**
SB3	19	4	2.00	0.228	0.187	2.00	-0.227
SB4	16	4	1.67	0.169	0.216	2.00	0.223
SB5	16	5	2.00	0.219	0.308	1.67	0.297**
SB6	16	5	1.83	0.188	0.192	2.00	0.022
SB7	19	3	1.67	0.114	0.185	1.83	0.391**
SM1	15	3	1.67	0.022	0.052	1.10	0.577**
SM2	19	0	1.00	0.000	0.000	1.00	N/A
SM3	16	1	1.17	0.019	0.018	1.07	-0.030
SLO1	19	4	1.67	0.192	0.189	1.66	-0.018
SLO2	16	4	1.83	0.131	0.227	1.78	0.433**
SLO3	15	5	1.83	0.102	0.224	1.78	0.554**
SF1	17	2	1.67	0.092	0.09	1.67	-0.020
SF2	20	2	1.33	0.109	0.085	1.33	-0.291
Mean	17.2	3.6	1.86	0.125	0.173	1.79	0.230
SD	3.2	1.4	0.48	0.070	0.083	0.40	0.330

* $P < 0.05$, ** $P < 0.001$.

Environmental correlates of inbreeding and genetic diversity

Estimates of the local inbreeding coefficient F_{IS} differed dramatically between populations north of LA ($\bar{F}_{IS} = 0.088$), and LA on south ($\bar{F}_{IS} = 0.341$). With two exceptions, outbreeding populations (where $F_{IS} < 0$) were only found north of LA, with the highest levels of

Table 4 Genetic variability of seven microsatellites in *Coccytrypes dactyliperda* from Ocean Beach and La Jolla: number of individuals genotyped (N), number of polymorphic loci (P), average number of alleles (n_a), average observed heterozygosity (H_O), average expected heterozygosity (H_E), average allelic richness (R_S), and fixation index between individuals and the local population (F_{IS}).

Site	N	P	n_a	H_O	H_E	R_S	F_{IS}
OB1	15	3	1.43	0.063	0.073	1.69	0.146
OB2	16	3	1.43	0.045	0.092	1.43	0.534**
OB3	16	4	1.57	0.092	0.112	1.55	0.178
OB4	15	4	1.57	0.067	0.135	1.57	0.516*
OB5	14	5	2.00	0.251	0.218	1.98	-0.156
OB6	16	5	1.86	0.152	0.181	1.84	0.163
OB7	14	5	1.71	0.136	0.162	1.68	0.168*
OB8	14	6	2.43	0.216	0.261	2.26	0.180*
OB9	15	3	1.43	0.161	0.189	1.43	0.151*
OB10	15	4	1.86	0.072	0.14	1.64	0.499*
LJ1	15	4	1.57	0.07	0.18	1.47	0.626**
LJ2	15	5	2.00	0.083	0.235	1.77	0.664**
LJ3	15	5	1.71	0.098	0.187	1.54	0.495*
LJ4	14	3	1.43	0.063	0.113	1.21	0.453*
LJ5	15	4	1.57	0.116	0.139	1.57	0.170
LJ6	15	3	1.43	0.07	0.082	1.42	0.148
LJ7	15	3	1.50	0.126	0.167	1.29	0.251
LJ8	15	4	1.71	0.074	0.154	1.65	0.531**
LJ9	15	5	2.00	0.097	0.213	1.81	0.554**
Mean		4.1	1.70	0.108	0.160	1.54	0.330
SD		0.9	0.27	0.055	0.052	0.25	0.224

* $P < 0.05$, ** $P < 0.001$.

outbreeding obtained in OX and SF (Table 3). The univariate relationship between F_{IS} and latitude appeared to be linear across most of the species range, and was statistically significant (linear regression; $P = 0.003$, slope = -0.094 ; Fig. 2). Exclusion of the SF populations at the northernmost edge of the study area did not affect these results ($P = 0.03$, slope = -0.087). F_{IS} was negatively correlated with precipitation and temperature PC1 (linear regression; $P = 0.001$, 0.06) and positively correlated with temperature PC2 ($P = 0.05$) but not elevation ($P = 0.8$). In a multiple regression of F_{IS} on latitude, annual precipitation, and the two temperature PCs, no predictors were statistically significant (all $P > 0.10$). Examination of all two- and three-factor sub-models showed that latitude and precipitation were each statistically significant when the other was not included in the model, while PC1 and PC2 were only significant when latitude and precipitation were both excluded. Thus, the decline of inbreeding with latitude does not seem to be related to temperature, but the data are inadequate to determine if precipitation may be an important correlate.

Levels of intrapopulation diversity (as estimated by R_S , H_O , H_S) displayed very different patterns than F_{IS} . None of the three diversity measures was significantly dependent on latitude or elevation in univariate or multivariate models. However, precipitation, PC1 and

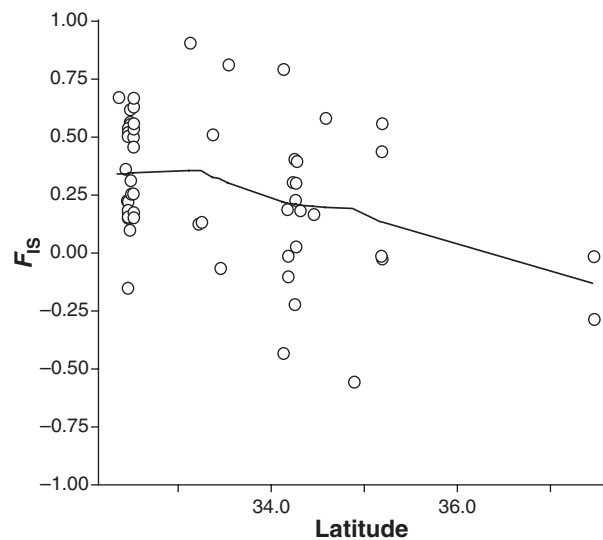


Fig. 2 Decline of F_{IS} with latitude. The line is a LOWESS smoother (20% span) for the entire data set.

PC2 were each statistically significant ($P \leq 0.05$) in multiple regressions for R_S , H_O and H_S . (Correlations with PC1 were negative in each case, while correlations with precipitation and PC2 were positive.) Thus, levels of genetic diversity within populations correlate with multiple environmental factors, but not with latitude.

Population structure

Statistically significant differentiation among populations was evident in all tests of genetic differentiation. In the hierarchical analysis, differentiation was high both within and among the 15 cities (metropolitan areas) in California ($F_{SC} = 0.434$, 95% CI = $0.226-0.541$; $F_{CT} = 0.310$, 95% CI = $0.142-0.431$). The highest pairwise F_{CT} values were generally found in contrasts with El Centro ($0.538-0.853$), and 79% of all pairwise F_{CT} contrasts were statistically significant (Table S1).

Differentiation on the smallest spatial scales was less pronounced. Mean pairwise F_{ST} was 0.070 and 0.112 within OB and LJ, respectively, and few pairwise F_{ST} estimates were statistically significant (three of 55 in OB after Bonferroni correction, and six of 45 within LJ; Table S2). Genetic divergence among populations did not fit a pattern of isolation by distance (IBD) at either site (Mantel test on log-transformed data, 10 000 randomizations, $P = 0.27$, $r = 0.12$). The genetic landscape visualizations for LJ and OB highlighted a number of patterns, including relatively high isolation for population LJ5 (Fig. 4a) and a 'crest' of relatively high genetic distance separating the western-most OB populations (OB1, OB2, OB3) from the remainder (visualized as the highest point in Fig. 4b).

At the larger statewide scale, IBD was statistically significant and relatively strong (Fig. 3; $P < 0.0001$, $r = 0.34$). Due to skewed distributions of both genetic and geographic distance, log transformation of both axes was necessary to better meet the assumptions of linear regression (compare Fig. 3a,b). For the log-transformed data, IBD patterns remained linear up to distances of 250 km (inferred from LOWESS smoother, Fig. 3b). The individual-based genetic landscape visualization of the statewide data set was dominated by a relatively flat topology in the northern half of the study range (VE and populations north), ramping down as genetic similarity increased south of VE (Fig. 4c). However, this discontinuity was due to skew in the untransformed data that differed between these two sets of populations, rather than any biological phenomenon. This discrepancy is highlighted in comparisons between the IBD slope for these two sets of populations before and after log transformation (straight solid and dashed lines in Fig. 3a vs. Fig. 3b). IBD slopes were identical in the northern

and southern portions of the study range after rescaling (Fig. 3b). The landscape visualization also identified genetic discontinuities that seem to represent populations with accelerated drift. For example, the depression between SF and San Luis Obispo emphasizes high genetic similarity across considerable distances, and a peak to the south highlights significant divergence of San Luis Obispo populations SLO1 and SLO2 from nearby SM. Although some smaller peaks may be associated with the overall landscape inflection point, there appears to be a real discontinuity between Avalon (on Catalina Island) and mainland California (LA, OS and LJ). The lowest point in Fig. 3c represents a high similarity among all populations in the El Centro area.

Discussion

Extensive population sampling and high levels of genetic variation allowed us to quantify population structure and patterns of inbreeding in *C. dactyliperda* across spatial scales ranging from 100 m to 850 km. Estimation of inbreeding coefficients confirmed that inbreeding represents the principle mating strategy of *C. dactyliperda*, with an average inbreeding coefficient of $F_{IS} = 0.27$ within populations (Tables 3 and 4). Although this is consistent with the unusual biology of this seed boring beetle, the tremendous variation in F_{IS} across populations surpassed all *a priori* expectations. Inbreeding coefficients (F_{IS}) varied dramatically among populations, from a high of 0.902 in OS to a low of -0.562 in OX (mean $F_{IS} = 0.27 \pm 0.33$; Table 3). Our survey of the literature did not uncover any other animal species with such high population variability in F_{IS} , suggesting that this may be the widest range ever reported. Our results, and those of a parallel study of the same species in Israel (D. Gottlieb *et al.*, unpublished data), support the hypothesis that these beetles have a stable 'mixed-mating' system, which span a range from completely inbred to highly outbred. The Israeli beetle populations also exhibited high levels of inbreeding ($F_{IS} = 0.306$) and considerable variability across populations (F_{IS} range: 0.125–0.620), although the range was more restricted than the California populations.

Although both the California and Israel studies supported similar conclusions regarding the mating system of *C. dactyliperda*, it is possible that so-called null alleles may have inflated our F_{IS} estimates. We attempted to minimize this problem by using species-specific primer sets, but could not rely on software programs that test for null alleles because they assume random mating. However, we note that laboratory experiments by D. Gottlieb *et al.* (unpublished data) provided insight into the mating behaviour of *C. dactyliperda* and explained how populations of these beetle could easily achieve the observed dramatic range of F_{IS} -values. Specifically, these data showed that *C. dactyliperda* females readily inbreed but prefer to outbreed given the opportunity (D. Gottlieb *et al.*, unpublished data; Fig. 1). Therefore, the F_{IS} -values

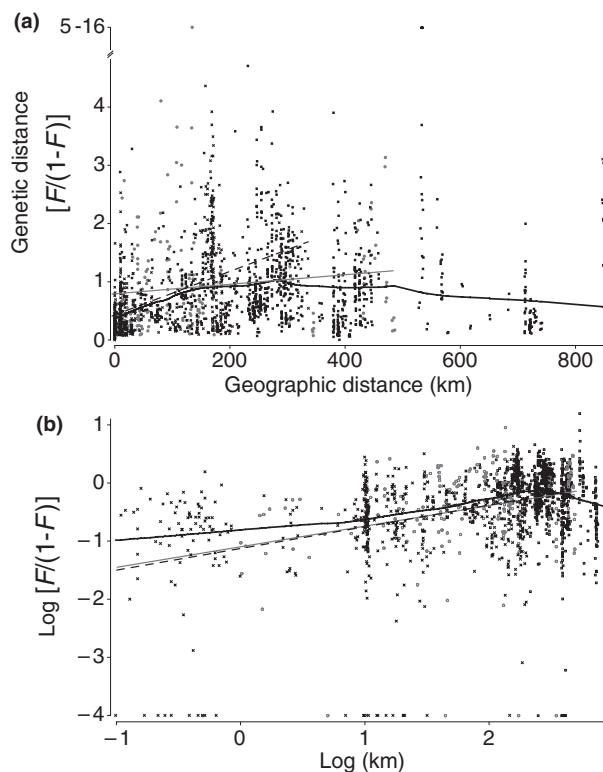


Fig. 3 Isolation by distance patterns for the entire data set (a) without and (b) with log transformation of both axes. For visualization purposes, the dashed regression line and crosses indicate contrasts between pairs of sites that are both south of Ventura. The solid grey line and grey circles indicate contrasts between pairs of sites that are both north of Ventura (inclusive of Ventura). Squares denote contrasts between two sites across this boundary, or with Avalon. The thick irregular line is a LOWESS smoother (20% span) for the entire data set.

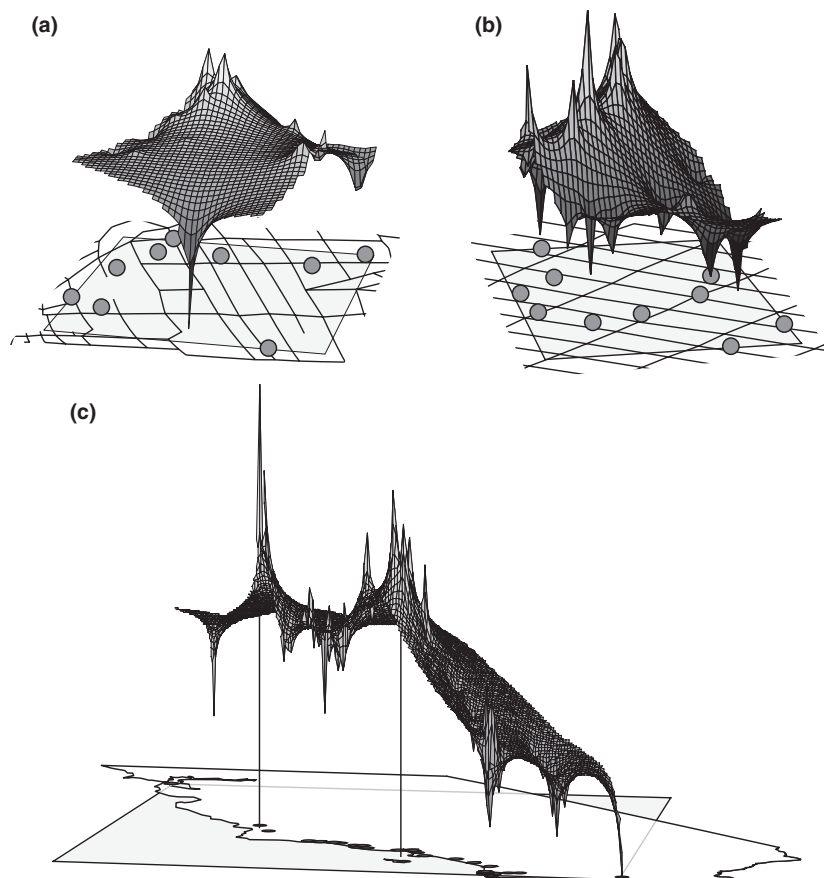


Fig. 4 Alleles In Space interpolation plots for (a) La Jolla, (b) Ocean Beach and (c) the entire data set. Positive 'peaks' represent high genetic discontinuities and negative peaks represent high genetic similarities. Interpolation surfaces for La Jolla and Ocean Beach are overlaid on local street maps. Vertical lines in the statewide map visualize the geographic locations of the highest peaks in the San Luis Obispo and Los Angeles areas.

found in the invasive California populations are entirely consistent with mating behaviour observed in the laboratory.

Latitudinal gradients

The high F_{IS} variability also allowed us to test for a linear relationship between inbreeding and latitude. Indeed, we found a significant negative relationship between F_{IS} and latitude ($P = 0.003$; $r^2 = 0.15$; Fig. 2). The correlation of F_{IS} with latitude turned out to be singular: no other diversity statistic measured in this study (P , n_e , H_E , H_S , or R_S) correlated with changes in latitude. This is consistent with inbreeding variation being driven by behavioural alteration of the *C. dactyliperda* reproductive strategy, rather than a side effect of population size or other demographic changes that might alter the amount of intrapopulation variation. The analysis suggests that environmental conditions that vary with latitude in California that also might affect the mating strategy of *C. dactyliperda*, although the multiple regression analysis discounts average precipitation and temperature as driving factors. Alternatively, lower F_{IS} -values in some regions (particularly those with negative F_{IS}) could

reflect strong inbreeding depression. The available data are insufficient to differentiate fully the relationships between environmental factors, natural selection via inbreeding depression, and decisions surrounding mate choice.

Our favoured hypothesis is that seed availability may drive much of the latitudinal trend in inbreeding. Two reviews of factors affecting seed production in plants (including palms), found an inverse relationship between the coefficient of variation in seed production and latitude (Koenig & Knops, 2000; Kelly & Sork, 2002). Greater variability in seed production at higher latitudes would mean less certainty of seed availability for the beetles at any given point in time. This may favour a delay in reproduction on the part of the beetle until a suitable food resource has been found in which to lay eggs, and would select for post-dispersal mating over predispersal. Further population sampling and experimentation will be necessary to test this hypothesis explicitly, but the evidence is at least consistent with the notion of a trade-off between early sexual maturity and successful dispersal. One relatively straightforward prediction of this hypothesis is that females in northern populations would tend to emerge prior to insemination,

while females in southern populations would emerge already inseminated by their males within the same seed. An alternative possibility is that, as multiple females can enter and breed in the same seed, there may be a latitudinal gradient in the ratio of the number of females to the number of available seeds. If seed availability is lower in more northerly latitudes, this could lead to increased numbers of females per available seed and, therefore, the probability of incidental outbreeding. In this case, the observed latitudinal gradient may be more a product of seed availability than a need for reproductive assurance, although these hypotheses are not mutually exclusive.

Regional population structure

Populations of *C. dactyliperda* displayed significant genetic structure and supported the hypothesis that human activity played the principal role in shaping the population structure of these beetles. Pairwise F_{CT} and R_{CT} estimates found high levels of differentiation between numerous pairs of populations within close geographic proximity (Table S1). In other instances, we found negligible F_{CT} - and R_{CT} -values between populations from very distant geographic locales (e.g. SF and LA). Both of these results indicate the possibility of multiple introductions across the state, although high values of differentiation can be misleading with haplodiploid inbreeders. Severe bottlenecks resulting in high population fixation indices may develop quickly over very short geographic distances (Tables S2 and S3), as only a single unmated female is necessary to found a population. Thus, high genetic differentiation values by themselves may not be reliable indicators of multiple independent introductions. On the other hand, low levels of differentiation found between geographically distant locales are harder to explain by natural dispersal processes or as byproducts of *C. dactyliperda* mating biology. For example, the extremely low levels of genetic differentiation between populations in SD, SLO, SM and SF argue that beetles in these populations shared a common source population. Similarly, populations in LG, AV and OX are separated by great distances, as well as a water barrier in the case of the Island of Avalon (AV), yet the F_{CT} - and R_{CT} -values remain low among them.

Interestingly, the areas with the highest levels of allelic diversity were located in El Centro and the port of San Pedro in LA (Table 3). The port of San Pedro is one of the largest entry ports on the west coast, while EC is a predominately agricultural area with a number of date palm orchards. Pairwise F_{CT} - and R_{CT} -values revealed high levels of differentiation between EC populations and all other sampled populations.

The A_{IS} genetic landscape shape interpolation plot of the state revealed a high genetic discontinuity between mainland California and AV, as well as with SB, VE and SLO (Fig. 4c). Although IBD patterns across the

state of California turned out to be significant (Fig. 3), this broad-scale pattern of IBD is unlikely to be the result of limited gene flow in continuous habitat. Founding events are likely to increase divergence among populations (Slatkin, 1977, 1993). This is most likely the case for metropolitan regions in California, which are separated by large stretches of unsuitable habitat lacking palm trees.

Local population structure

Densely sampled neighbourhood populations in OB and LJ provided insight for understanding the scale at which genetic differentiation of *C. dactyliperda* occurs in urbanized habitat. We found high levels of statistically significant genetic differentiation in both OB and LJ between populations separated by only a few city blocks (Tables S2 and S3). In LJ, the highest pairwise genetic differentiation values even approached the F_{CT}/R_{CT} -values found between some metropolitan regions in the state (Table S1). We suspect that the high F_{ST}/R_{ST} -values reflect a combination of the beetles haplodiploid inbreeding biology and patterns of localized human disturbance. Theoretically, a single *C. dactyliperda* female can establish a new population by mating with her own haploid son. Such a founding event would surely result in a dramatic shift in allelic frequencies through genetic drift. In addition, the palm seeds comprising the beetle's habitat in urbanized areas tend to be extremely disturbed. On a number of occasions, we noted almost complete removal of seeds around palms as yard waste (Holzman & Kelley, personal observation). Removing high numbers of infested seeds would result in local population extinctions, or at least severe bottlenecks. In future studies, the relative importance of founder events vs. seed removal bottlenecks could be quantified by also sampling patterns areas with little or no human disturbance.

The effects of urbanized disturbance were also seen in the genetic landscape visualizations, in which we detected apparent barriers to gene flow over small spatial scales (< 100 m). For example, the high differentiation separating OB 1, 2 and 3 from the remainder of the populations corresponds to a heavily trafficked North-South road in the neighbourhood (Fig. 4b), similar to previous studies that have found genetic isolation caused by major roads (e.g. Mader, 1984; Keller & Largiadèr, 2003; Vandergast *et al.*, in press). Isolation by distance trends both within and between OB and LJ may suggest the development of broader-scale limitations to gene flow. The Phoenix palms in OB and LJ can have been occupied for a maximum of 76 and 93 years, respectively. In favourable conditions, *C. dactyliperda* can have up to five generations per year (Bar-Shalom & Mendel, 2003), which would result in an estimated number of 380–465 generations since introduction. Although 100 years is a relatively short-time frame to observe the development of IBD patterns and strong genetic

differentiation, it seems reasonable given the generation time of these beetles.

Conclusion

Invasive species can experience dramatic shifts in allelic frequencies through both drift and selection. Severe bottlenecks followed by rapid population expansions typically accompany invasions, and individuals often encounter novel selection pressures. The remarkable development of a clear latitudinal cline in breeding system in *C. dactyliperda* over a short evolutionary time frame illustrates the potential evolutionary importance of mating behaviour in these situations. We suggest that seasonal seed availability may best explain the observed cline, although we cannot completely rule out historical artefacts of the colonization sequence, or adaptation to other factors. Because *C. dactyliperda* is amenable to both field and laboratory studies, future studies may further explore the impact of seed availability on inbreeding, as well as latitudinal differences in pre- vs. post-dispersal mating.

Acknowledgments

We thank Oliver Ryder and the Genetic Division at CRES for use of their ABI 3100 automated sequencer and Forest Rower for use of the Microchemical Core Facility at SDSU. We also thank N. Marshall, E. Lewallen and C. Roth for their comments and observations on this study. Finally, we thank our anonymous reviewer for contributing excellent and insightful comments on our manuscript, as well as the JEB editorial staff with their help and patience during the review process.

References

- Atkinson, T.H. & Peck, S.B. 1994. Annotated checklist of the bark and ambrosia beetles (Coleoptera: Platypodidae and Scolytidae) of tropical southern Florida. *Florida Entomol.* **77**: 313–329.
- Bar-Shalom, O. & Mendel, Z. 2003. Population size and distribution of the stone palm beetle *Coccotrypes dactyliperda* (Scolytidae) in Israel in relation to the date palm cultivated areas. *Alon. Hanotea.* **57**: 537–540.
- Bell, G. 1982. *The Masterpiece of Nature: the Evolution and Genetics of Sexuality*. Croom Helm, London, UK.
- Berg, P.R., Dawson, D.A., Pandhal, J., Kirkendall, L.R. & Burke, T. 2003. Isolation and characterization of microsatellite loci from two inbreeding bark beetle species (*Coccotrypes*). *Mol. Ecol. Notes* **3**: 270–273.
- Bierzychudek, P. 1987. Patterns in plant parthenogenesis. *Experientia* **55**(Suppl.): 197–217.
- Carol, E.L. 2002. Evolutionary genetics of invasive species. *Trends Ecol. Evol.* **17**: 386–391.
- Carruthers, R.I. 2003. Invasive species research in the United States Department of Agriculture-Agricultural Research Service. *Pest Manag. Sci.* **59**: 827–834.
- Cohen, A.N. & Carlton, J.T. 1998. Accelerating invasion rate in a highly invaded estuaries. *Science* **279**: 555–558.
- Darvill, B., Ellis, J.S., Lye, G.C. & Goulson, D. 2006. Population structure and inbreeding in a rare and declining bumblebee *Bombus muscorum* (Hymenoptera: Apidae). *Mol. Ecol.* **15**: 601–611.
- Excoffier, L., Laval, G. & Schneider, S. 2005. ARLEQUIN 3.0: an integrated software package for population genetics data analysis. *Evol. Bioinform. Online* **1**: 47–50.
- Glesener, R.R. & Tilman, D. 1978. Sexuality and components of environmental uncertainty – clues from geographic parthenogenesis in terrestrial animals. *Am. Nat.* **112**: 659–673.
- Goudet, J. 1995. FSTAT 1.2: a computer program to calculate F-statistics. *J. Hered.* **86**: 485–486.
- Haack, R.A. 2001. Scolytidae (Coleoptera) at US Ports of Entry. 1985–2000 *Integrated Pest Management Reviews* **6**: 253–282.
- Herfs, A. 1950. Studien an den Steunnussborckenkäfer *Coccotrypes tangeranus* Eggers. *Hofchenbriefe Wissenschaft. Praxis* **3**: 3–31.
- Holway, D.A. 1999. Competitive mechanisms underlying the displacement of native ants by the invasive argentine ant. *Ecology* **80**: 238–251.
- Human, K.G. & Gordon, D.M. 1997. Effects of argentine ants on invertebrate biodiversity in Northern California. *Conserv. Biol.* **11**: 1242–1248.
- Jensen, J., Bohonak, A. & Kelley, S.T. 2005. Isolation by distance web service. *BMC Genet.* **6**: 13.
- Jordal, B.H., Beaver, R.A. & Kirkendall, L.R. 2001. Breaking taboos in the tropics: incest promotes colonization by wood-boring beetles. *Glob. Ecol. Biogeogr.* **10**: 345–357.
- Jordal, B.H., Normark, B.B., Farrell, B.D. & Kirkendall, L.R. 2002. Extraordinary haplotype diversity in haplodiploid inbreeders: phylogenetics and evolution of the bark beetle genus *Coccotrypes*. *Mol. Phylogenet. Evol.* **23**: 171–188.
- Keller, I. & Lurgiader, C.R. 2003. Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. *Proc. R. Soc. Lond. B* **270**: 417–423.
- Keller, L.F. & Waller, D.M. 2002. Inbreeding effects in wild populations. *Trends Ecol. Evol.* **17**: 230–241.
- Kelly, D. & Sork, V.L. 2002. Mast seeding in perennial plants: why how where? *Annu. Rev. Ecol. Syst.* **33**: 427–447.
- Kirkendall, L. 1993. Ecology and evolution of biased sex ratios in bark and ambrosia beetles. In: *Evolution and Diversity of Sex Ratio in Insects and Mites* (D.L. Wrensch & M.A. Ebbert, eds), pp. 234–345. Chapman & Hall, New York, NY, USA.
- Koenig, W.D. & Knops, J.M. 2000. Patterns of annual seed production by northern hemisphere trees: a global perspective. *Am. Nat.* **155**: 59–69.
- Krakowski, J., Aitken, S.N. & El-Kassaby, Y.A. 2003. Inbreeding and conservation genetics in whitebark pine. *Conserv. Genet.* **4**: 581–593.
- Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. & Bazzaz, F.A. 2000. Biotic invasions: causes epidemiology global consequences and control. *Ecol. Appl.* **10**: 689–710.
- Mader, H.J. 1984. Animal habitat isolation by roads and agricultural fields. *Biol. Conserv.* **29**: 81–96.
- Mantel, N. 1967. Detection of disease clustering and a generalized regression approach. *Cancer Res.* **27**: 209–220.
- Mardulyn, P. & Milinkovitch, M.C. 2005. Inferring contemporary levels of gene flow and demographic history in a local population of the leaf beetle *Goniocetena olivacea* from mitochondrial DNA sequence variation. *Mol. Ecol.* **14**: 1641–1653.

- Miller, M.P. 1998. **TFPGA**: Tools for Population Genetic Analyses for Windows. Arizona State University, Tempe, AZ, USA.
- Miller, M.P. 2005. Alleles In Space **AIS**: computer software for the joint analysis of interindividual spatial and genetic information. *J. Hered.* **96**: 722–724.
- Mitton, J.B. 1997. *Selection in Natural Populations*. Oxford University Press, Oxford, UK.
- Mooney, H.A. & Cleland, E.E. 2001. The Evolutionary impact of invasive species. *Proc. Natl Acad. Sci. USA* **98**: 5446–5451.
- Mousadik, A. & Petit, R.J. 1996. High level of genetic differentiation for allelic richness among populations of the argan tree [*Argania spinosa* L. Skeels] endemic to Morocco. *Theor. Appl. Genet.* **92**: 832–839.
- Mun, J., Bohonak, A.J. & Roderick, G.K. 2003. Population structure of the pumpkin fruit fly *Bactrocera depressa* (Tephritidae) in Korea and Japan: Pliocene allopatry or recent invasion? *Mol. Ecol.* **12**: 2941–2951.
- Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**: 583–590.
- Nico, L.G. & Fuller, P.L. 1999. The evolutionary impact of invasive species. *Fisheries* **24**: 1–27.
- Peer, K. & Taborsky, M. 2005. Out-breeding depression but no inbreeding depression in haplodiploid *Ambrosia* Beetles with regular sibling mating. *Evolution* **59**: 317–323.
- Potter, D.A. & Held, D.W. 2002. Biology and management of the Japanese beetle. *Annu. Rev. Entomol.* **47**: 175–205.
- Pritchard, J.K., Stephens, M. & Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945–959.
- Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* **145**: 1219–1228.
- Rousset, F. & Raymond, M. 1995. Testing heterozygote excess and deficiency. *Genetics* **140**: 1413–1419.
- Slatkin, M. 1977. Gene flow and genetic drift in a species subject to frequent extinctions. *Theor. Popul. Biol.* **12**: 253–262.
- Slatkin, M. 1993. Isolation by distance in equilibrium and non-equilibrium populations. *Evolution* **47**: 264–279.
- Thomson, D. 2004. Competitive interactions between the invasive European Honey bee and native Bumble bees. *Ecology* **85**: 458–470.
- Vandergast, A.G., Lewallen, E.A., Deas, J., Bohonak, A.J., Weissman, D.B. & Fisher, R.N. Loss of genetic connectivity and diversity in urban microreserves in a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: *Stenopelmatus* “santa monica”). *J. Insect Conserv.*, in press.
- Velleman, P.F. 1997. **DATADESK 6.2** for Macintosh Data Description. Data Description, Inc., Ithaca, NY, USA.
- Vitousek, P.M., D’Antonio, C.M., Loope, L.L., Rejmanek, M. & Westbrooks, R. 1997. Introduced species: a significant component of human-caused global change. *N. Z. J. Ecol.* **21**: 1–16.
- Wadsworth, R.A., Collingham, Y.C., Willis, S.G., Huntley, B. & Hulme, P.E. 2000. Simulating the spread and management of alien riparian weeds: are they out of control? *J. Appl. Ecol.* **37**: 28–38.
- Waples, R.S. & Gaggiotti, O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Mol. Ecol.* **15**: 1419–1439.
- Werren, J.H. 1993. The natural history of inbreeding and outbreeding. In: *The Evolution of Inbreeding in Haplodiploid Organisms* (N.W. Thornhill, ed.), pp. 42–49. University of Chicago Press Chicago, Chicago, IL, USA.
- Wilcove, D.S., Rothstein, D., Dubow, J., Phillips, A. & Losos, E. 1998. Quantifying threats to imperiled species in the United States. *Bioscience* **48**: 607–615.
- Williamson, M.H. 1998. *Biological Invasions*. Chapman & Hall, London, UK.
- Wood, S.L. 1982. *The Bark and Ambrosia Beetles of North and Central America (Coleoptera: Scolytidae) A Taxonomic Monograph*. Brigham Young University Press, Provo, UT, USA.
- Work, T.T., McCullough, D.G., Cavey, J.F. & Komsa, R. 2005. Arrival rate of nonindigenous insect species into the United States through foreign trade. *Biol. Invasions* **7**: 323–332.
- Wright, S. 1943. Isolation by distance. *Genetics* **28**: 114–138.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Table S1 Pairwise comparisons of F_{CT} (bottom half) and R_{CT} (top half) between metropolitan regions of California.

Table S2 Pairwise comparisons of F_{ST} (bottom half) and R_{ST} (top half) between OB populations.

Table S3 Pairwise comparisons of F_{ST} (bottom half) and R_{ST} (top half) between LJ populations.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

Received 12 October 2008; revised 16 January 2009; accepted 2 February 2009