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ORIGINAL ARTICLE

Diversity in insect seed parasite guilds at large geographical scale: the roles of host specificity and spatial distance

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ABSTRACT

Aim Host specificity within plant-feeding insects constitutes a fascinating example of natural selection that promotes inter-specific niche segregation. If specificity is strong, composition of local plant parasitic insect guilds is largely dependent on the presence and prevalence of the preferred hosts. Alternatively, if it is weak or absent, historic and stochastic demographic processes may drive the structuring of insect communities. We assessed whether the species composition of acorn feeding insects (*Curculio* spp. guilds) and their genetic variation change geographically according to the local host community.

Location An 800 km transect across California, USA.

Methods We used DNA taxonomy to detect potential *Curculio* cryptic speciation and assessed intra-specific genetic structure among sampling sites. We monitored larval performance on different hosts, by measuring the weight of each larva upon emerging from the acorn. Our phylogenetic and spatial analyses disentangled host specificity and geographical effects on *Curculio* community composition and genetic structure.

Results DNA taxonomy revealed no specialized cryptic species. Californian *Curculio* spp. were sister taxa that did not segregate among *Quercus* species or, at a deeper taxonomic level, between red and white oaks. *Curculio* species turnover and intra-specific genetic differentiation increased with geographical distance among localities irrespective of local oak species composition. Moreover, larval performance did not differ among oak species or acorn sizes when controlling for the effect of the locality.

Main conclusions Historical processes have contributed to the structuring of acorn weevil communities across California. Trophic niche overlapped among species, indicating that ecologically similar species can co-exist. Acorn crop inter-annual variability and unpredictability in mixed oak forests may have selected against narrow specialization, and facilitated co-existence by means of an inter-specific time partitioning of the resources. Wide-scale geographical records of parasitic insects and their host plants are necessary to understand the processes underlying species diversity.

Keywords
acorn, California, *Quercus* spp., seed-feeding insects, spatial autocorrelation, species turnover

INTRODUCTION

The different potential factors underlying species assemblages have been widely debated but still remain a current topic in ecology and, particularly, in plant–insect interactions research. The Competitive Exclusion Principle states that multiple species cannot utilize the same limiting trophic resources indefinitely. Thus, selection on each species results from inter-specific specialization that guarantees some portion of the resource is acquired (Hardin, 1960). In contrast,

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the neutral Theory of Biodiversity assumes that competing species are ecologically similar, and predicts that the structure of their communities will depend on historical demographic processes like extinction/migration dynamics (Bell, 2001). The Co-existence Theory (Chesson, 2000) supports the Neutral Theory of Biodiversity proposing mechanisms to explain how co-existing competing species can sustainably maintain an overlapping trophic niche.

Most previous research aiming to separate the contribution of competition and historical factors on species assemblages has been limited to similar species, usually from a few or single sampling localities (see Skoracka & Kuczyński, 2012 for a review on insect herbivorous guilds), which may neglect historical factors operating at a larger scale. We aim to fill this gap by sampling acorn parasitic insects *Curculio* spp. captured within multiple host species across a wide geographical scale in the state of California.

Insect parasitism on plants is a good example of how intimate species interactions and competition for limited resources can drive specialization (e.g. Cook *et al.*, 2002). Many parasitic insects carry morphological, behavioural and physicochemical traits adapted to the characteristics of their host plants (i.e. phenology, leaf or seed morphology, physicochemical defences) (Pearse & Hipp, 2009; Yguel *et al.*, 2011). Trophic specialization drives phylogenetic specificity, which has a variable taxonomic spread: from taxa that feed on plants of the same family or genus to extreme specialists that exploit only one species (reviewed in Barrett & Heil, 2012).

The degree to which specificity is possible within parasitic insects is dependent on the strength of homogenizing and differentiating forces across their range. Specificity may start at the intra-specific level, when local adaptation to different hosts drives divergence between populations of the same parasite species (Thompson, 1999; Drummond *et al.*, 2010). Populations separated in space, with reduced gene flow homogenizing genetic variance, have a greater likelihood of diverging, with taxa splitting into new species that optimize their performance on the preferred hosts and so increasing their relative fitness (Sword *et al.*, 2005). Nevertheless, differentiation is not always morphologically evident, and may require molecular techniques to discern species (i.e. specialized cryptic species in Murray *et al.*, 2007; review in Barrett & Heil, 2012). Regional scale records of parasitic insects and their host plants could identify the degree to which host specificity drives regional species diversity, while accounting for the influence of geographical separation and climatic variance that may additionally drive local adaptation.

We chose Californian acorn weevils as a case-study because California is a biodiversity hotspot with physical barriers, heterogeneous habitats, and climatic conditions that have dramatically shaped species diversification, distribution and genetic structure (Calsbeek *et al.*, 2003; Davis *et al.*, 2008). Weevils (Coleoptera: Curculionidae) parasitize oak acorns worldwide (Bonal *et al.*, 2011; Toju & Fukatsu, 2011; Govindan *et al.*, 2012) and [like most of their endemic host oaks (Nixon, 2002)] are widely distributed across California (Gibson, 1969). With such an extensive distribution over a climatically and topographically diverse region, independent geographical effects may have played a significant role in structuring weevil communities. Nevertheless, previous weevil studies have sought only ecological explanations for species structuring. Govindan *et al.* (2012) reported inter-specific segregation and showed that weevils that fed on acorns of their preferred oak species had a greater survival likelihood. Other authors have hypothesized that inter-specific diversification of weevils has been driven by body size adaptation to the size of the acorns exploited (Hughes & Vogler, 2004a; Bonal *et al.*, 2011). However, in all cases host records come from taxonomic oriented articles (Gibson, 1969), or population-level studies carried out at a small spatial scale examining only a few of the potential host species.

Our main objective was to test *Curculio* spp. host specificity after accounting for variation in the geographical structure of the parasite species prevalence, genetic differentiation, and performance. Weevils were collected from eight oak species from the two major sections (*Erythrobalanus* and *Leucobalanus*) within the genus *Quercus*. We sampled the majority of hosts and parasite geographical ranges and performed DNA-based species delimitation of weevils to detect potential host-specialized cryptic taxa. Specifically, (1) we studied host species specialization in acorn weevils by assessing whether species turnover and intra-specific genetic differentiation between localities depended on host species similarity or simple spatial proximity; (2) we studied acorn size specialization by comparing the size of the acorns exploited by the different weevil species within the same locality; (3) Finally, we examined weevil weight upon emerging from an acorn to analyse the potential impact of host–specific ability on weevil performance.

MATERIALS AND METHODS

Study area and species

From late September to mid October 2010 we sampled at a total of 29 localities widespread over the state of California (north–south and east–west ranges of 805 and 531 km respectively) (see Appendix S1 in Supporting Information; Fig. 1). Each site was georeferenced and we sampled all oak species present, when acorn availability and spatio-temporal variation in crop production (Koenig *et al.*, 1994) permitted. We collected acorns from the most widespread oak species of California, as well as some narrowly distributed endemics, including both red oaks, section *Erythrobalanus* (*Q. agrifolia*, *Q. kelloggii*, *Q. wislizenii*) and white oaks, section *Leucobalanus* (*Q. lobata*, *Q. douglasii*, *Q. engelmanii*, *Q. berberidifolia*, *Q. cornellius-mulleri*) (see Appendix S1).

Weevils (*Curculio* spp. Coleoptera, Curculionidae) are the main pre-dispersal acorn predators and may attack more than 80% of the crop (Gibson, 1969; Bonal *et al.*, 2007; Espelta *et al.*, 2008). Predation occurs by parasitism, when

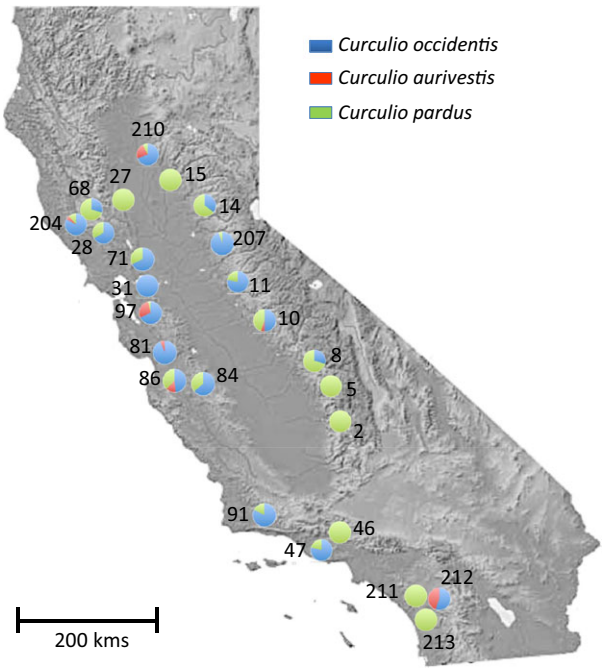

Figure 1 Map of California with the locations of the 25 sampling sites where at least nine weevils were sampled. The proportions of each species (*Curculio pardus*, *C. occidentis* and *C. aurivestis*) at each site are shown. Numbers correspond to population codes described in Appendix S1.

Curculio spp. females oviposit into the acorns, where larvae feed on the cotyledons as they develop. To date, three species of acorn weevils have been recorded in California (*C. pardus*, *C. occidentis* and *C. aurivestis*) with most of their populations located within the study area, spreading marginally to the north and east (Gibson, 1969). To confirm that they do not spread further east, we included in the analyses weevil larvae collected in Utah (37°02′45″, 112°43′23″).

Adult weevils were collected by gently shaking the oak branches over a white blanket and larvae were collected from infested acorns. At the laboratory facilities of the University of California Los Angeles (UCLA) adults were identified to the species level following Gibson (1969). Infested acorns were separated into plastic dishes, and kept at 20 °C to provide identical development conditions to all larvae. After emerging, larvae were weighed and then stored in tubes filled with 99% ethanol for later DNA extraction.

DNA extraction and sequencing

We selected 672 weevils for molecular analyses, balancing the number of individuals between host-oak species and localities. We included 14 adults representing the three species of Californian acorn *Curculio*, the rest (90% of the samples) were larvae to be further identified by means of DNA taxonomy (see Pinzon-Navarro *et al.*, 2010 for a similar procedure). DNA was extracted from insect tissue according to the Aljanabi & Martínez (1997) salt extraction protocol.

Individuals were genotyped by amplifying two mitochondrial genes, encoding cytochrome oxidase I (cox1) and cytochrome B (cytb). We used the primers C1-J-2183 (Jerry) and L2- N-3014 (Pat) for the first and the universal primers CB1 and CB2 for the second. In addition, we amplified a fragment of the nuclear gene encoding elongation factor 1α (EF-1α) using EF1-R and EF1-F primers [see Hughes & Vogler, 2004b for details on the polymerase chain reaction (PCR) conditions for the three genes]. Sequence chromatograms were assembled and edited using Sequencher 4.6 (Gene Codes Corp., Ann Arbor, MI, USA). The sequences of the three genes (cox1, cytb and EF-1α) were trimmed to 711, 413 and 581 base pairs, respectively, to reduce the proportion of missing data. In the case of the nuclear gene EF-1α some sequences contained gaps in the intron region.

Species delimitation and phylogenetic analyses

We pooled the cox1 sequences of all individuals to delimit the different species according to the generalized mixed Yule-coalescent (GMYC) model (Pons *et al.*, 2006) implemented in R package 'splits', in which we used the 'single threshold' option (Pons *et al.*, 2006). We built a maximum likelihood (ML) tree including one copy of each haplotype applying a GTR+I+Gamma substitution model – according to the results of jModeltest 0.1.1 (Posada, 2008). The gall feeding weevil *C. pyrrhoceras* was used as outgroup, as it presents the greatest divergence to the other *Curculio* species for the three genes analysed (see Hughes & Vogler, 2004b). The analysis was performed with RAxML 7.0.4 (Stamatakis, 2006) and the resulting tree was made ultrametric under a molecular clock model in paup*4.0b10 (Swofford, 2002) with the parameters estimated from the ML search. The GMYC model tracks the tree branching rates and detects the transition from among-species to within-population branching patterns, delimiting 'independently evolving' mtDNA clusters. These clusters are called GMYC (putative) species and, if they include sequences from known Linnean species, they may serve to differentiate otherwise indistinguishable specimens like weevil larvae (for which there are no morphological keys) (Pinzon-Navarro *et al.*, 2010).

One individual per GMYC group was chosen for a more detailed phylogenetic analysis based on three genes (cox1, cytb and EF-1α). Our main objective was to assess the phylogenetic relationships among the Californian weevils to investigate whether host shifts may be involved in species splitting. To do so, we pooled the Californian sequences with those of another 17 species of American and European *Curculio* (Hughes & Vogler, 2004a). The three genes were aligned separately with ClustalW (Thompson *et al.*, 1994). In the case of the nuclear EF-1α we used the gap opening and gap extension penalties provided by default by ClustalW (15 and 6.66 respectively), and visual inspection of the alignment showed that those values were accurate. Next, all

genes were concatenated, realigned and our final sequence data file was visually revised to make sure that there were no errors. The gall eating *C. pyrrhoceras* was the outgroup in all phylogenies (see Hughes & Vogler, 2004b).

We built phylogenetic trees following two methods (ML and Bayesian inference) and assessed whether they retrieved the same tree topology. We calculated the best-fit models of nucleotide substitution for each of the three genes according to the Akaike information criterion using jModeltest 0.1.1 (Posada, 2008). Maximum likelihood analyses were performed in RAxML 7.2.6 (Stamatakis, 2006) and phyml 3.0 (Guindon & Gascuel, 2006). In RAxML three partitions were set (one for each gene) and 10 independent searches conducted. phyml was additionally used to assess the repeatability of the topology and also because it allows calculating the approximate likelihood-ratio test for branch support, which is a good alternative to nonparametric bootstrap (Guindon & Gascuel, 2006). Bayesian inference analyses were performed with MrBayes 3.2 (Ronquist *et al.*, 2012). We used the same partitions as we used in the ML tree (RAxML), applying a nucleotide substitution model specific to each gene. Two parallel runs of 2 million generations each were conducted using one cold and two incrementally heated Markov chains ($\Lambda = 0.2$), sampling every 1000 steps. We first checked one of the standard convergence diagnostics implemented in MrBayes and then assessed the average standard deviation of the split frequencies to deduce that the Markov chain had reached stationarity. After 500,000 generations, the average standard deviation of the split frequencies stabilized in values close to zero (0.001). Hence, phylogenetic trees were summarized using the all-compatible consensus command with 25% burn-in.

Intra-specific genetic structure

We analysed inter-population genetic differentiation in those species (*C. pardus* and *C. occidentis*) that had a sufficient number of specimens per sampling locality (see below the choice criteria). We performed analyses of the molecular variance (AMOVAs) using Arlequin software (Excoffier *et al.*, 2005) and also tested whether there was any geographical pattern in the population genetic structure using Samova 1.0 (Dupanloup *et al.*, 2002). This method identifies the optimal grouping option (K) that maximizes the among-group component (FCT) of the overall genetic variance. We defined the number of populations (K) and ran 100 simulated annealing processes. We simulated different numbers of populations, ranging from $K = 2$ to 19, to determine the best population clustering option.

Curculio intra-specific genetic dissimilarities among hosts and localities

We performed intra-specific analyses on *C. pardus* and *C. occidentis* (*C. aurivestis* samples did not reach a sufficient number per site). We included only those localities in which there were sequences for at least four individuals per species (see Papadopoulou *et al.*, 2011 for a similar approach). Above this threshold we confirmed that there was no effect of sample size on either genetic (*C. pardus*: $r = 0.31$, $P = 0.17$, $n = 20$; *C. occidentis*: $r = 0.09$, $P = 0.68$, $n = 19$) or nucleotide diversity (*C. pardus*: $r = 0.21$, $P = 0.36$, $n = 20$; *C. occidentis*: $r = 0.001$, $P = 0.99$, $n = 19$). We used Arlequin 3.1 (Excoffier *et al.*, 2005) to compute genetic dissimilarities by assessing the raw average number of differences among populations (Nei's D) in the mitochondrial gene cox1. DNA microsatellites (nuclear DNA) have yet to be developed for these species and, although they can provide finer resolution in genetic analysis, in other *Curculio* spp. mitochondrial markers have detected population structure at scales of just a few kilometres and distinguished host-adapted morphotypes (Toju & Sota, 2006; Toju *et al.*, 2011). Host-oak dissimilarities were calculated using Bray-Curtis index on the number of *Curculio* individuals sampled on each oak species and its correlation with intra-specific genetic dissimilarities was analysed controlling for the effect of the Euclidean geographical distance between localities using partial Mantel tests as implemented in the R package '*ecodist*' (Goslee & Urban, 2007).

Local oak community composition and *Curculio* species turnover among sites

Due to variable insect availability it was not always possible to balance weevil sample size across sites and *Quercus* species, hence we included those 25 localities with nine or more individuals (see Appendix S1). Above that number we found no significant effect of sample size on either the number of species (Spearman correlation: $r = 0.34$, $P = 0.14$, $n = 25$) or species α-diversity (Spearman correlation: $r = 0.10$, $P = 0.60$, $n = 25$) collected at a site. Further, in 16 localities in which sample size was > 18, we calculated the mean rarified number of species standardized first for 9 and then for 18 individuals, and found no significant differences between the two estimates (ANOVA: $F_{1,30} = 0.57$; $P = 0.45$). Species diversity and richness measures were calculated using the R package 'vegan' (Oksanen *et al.*, 2011). Statistical analyses were performed using R (R Development Core Team, 2012).

We examined the influence of host-oak communities on weevil species compositional dissimilarity. Pairwise *Curculio* species turnover among localities was assessed with the Bray–Curtis index. This index is calculated using species presence/absence and relative abundance, making it less affected by low species numbers. We measured the correlation between *Curculio* spp. and host-oak similarities with a partial Mantel test (10,000 permutations) using the Euclidean geographical distance among localities as a control for potential spatial autocorrelation effects (Koenig, 1999). We ran this analysis using the R package 'ecodist' (Goslee & Urban, 2007).

Curculio inter-specific segregation according to host size

We assessed whether acorns were partitioned by size among the larvae of *C. pardus* and *C. occidentis*. *Curculio aurivestis* was not included due to low sample sizes. The raw weight of infested acorns is an unreliable estimate of acorn size, as weight varies with the amount of cotyledon eaten by the larvae inside. Instead, we used linear dimensions of each acorn (length and width to the nearest 0.01 mm) to estimate acorn mass using the formula detailed in Bonal *et al.* (2007). In those localities where both weevil species co-existed and at least three larvae of each species were collected, we compared the size of the acorns exploited by each with a paired Student's *t*-test. As body size affects the size of the acorns used (Bonal *et al.*, 2011), we also compared *C. pardus* and *C. occidentis* larval weight with a paired Student's *t*-test.

Curculio performance according to host species identity and host seed size

We estimated *Curculio* performance by recording the larval weight (to the nearest 0.1 mg) when they emerged from infested acorns. Larval weight is a key life history trait in most insects and a good fitness proxy. Within *Curculio* weevils larval weight determines to a large extent survival likelihood and potential fecundity (Desouhant *et al.*, 2000; Bonal *et al.*, 2012). We dried all the infested acorns at 80 °C for 48 h before opening them 1 month after the last larva had emerged. We found that the cotyledons were never depleted within our samples, so any difference in larval weight would be the due to the nutritional quality of the acorn rather than to food constraints.

We used an ANCOVA to test the effect of the host-oak species (fixed factor) and acorn mass (covariate) on larval weight (dependent variable). Sampling locality was included as a random effect because insect body size has been shown to be susceptible to changes at geographical scale due to environmental differences among localities (Mousseau & Roff, 1989). We ran this analysis first examining all weevils, and then used just those collected on the most frequently sampled oak species (*Q. lobata*) to remove any potential confounding effect of host species identity. Statistical analyses were performed with STATISTICA 7.0 (Statsoft, Inc Tulsa, OK, USA).

RESULTS

DNA-based weevil species delimitation and phylogenetic analyses

A total of 540 cox1 sequences from adult weevils and larvae had the necessary length to be included in the analyses (of these 529 were collected in California and 11 in Utah). We did not get sequences for the remaining 132 individuals (20%), either due to PCR issues, or because the sequences obtained were not long enough. These 540 sequences were collapsed into 133 different haplotypes (GenBank accession numbers Banklt1883487 KU378235 - KU378367) that were used to build the ultrametric clock-constrained ML phylogeny subjected to the GMYC analysis, which grouped the sequences in four clusters corresponding to distinct putative species. Three of these clusters included sequences obtained from both adults and larvae collected in California. All clusters corresponded to just one previously named species (*C. pardus*, *C. aurivestis* or *C. occidentis*). All adult species assignments based on morphological characters matched the species assignment based on the GMYC cluster, confirming the reliability of our genetic methods and ability to accurately determine all larvae to the species level. The fourth cluster corresponded to the weevil larvae collected in Utah and could not be identified because their sequences did not group with any acorn *Curculio* species available in GenBank; they were named GMYC 51 (Fig. 2).

The three phylogenies built on the combined three genes set (mitochondrial cox1 and citb; nuclear EF-1α) retrieved the same topology (Fig. 2). The tree shows a clear division between North American and European species, which form different clades with a very strong branch support. The Californian acorn weevil species constitute a subclade nested in the American clade (Fig. 2). There is strong support for a sister species relationship between *C. aurivestis* and *C. occidentis*, and comparatively low support for a clade containing *C. pardus*, *C. aurivestis* and *C. occidentis*, indicating that the relationship of *C. pardus* to the other two species is less certain.

Host specificity and species turnover

The phylogenetic tree shows that host shifts between oak species or sections (red and white oaks) were not involved in the speciation of the Californian acorn *Curculio* spp. (Fig. 2). *Curculio pardus* and *C. occidentis* were present on all *Quercus* spp. sampled with the exception of *C. occidentis* on *Q. cornellius-mulleri*. The scarce *C. aurivestis* was not found on *Q. wislizenii*, *Q. kellogii* and *Q. berberidifolia.*

Species distribution patterns were defined by geographical restriction and not host tree assembly (Fig. 1). The Mantel test showed that geographically more distant populations harboured more dissimilar *Curculio* spp. communities ($r = 0.14$, $P = 0.03$), but host oak similarity among localities was non-significant when examined at the species ($r = 0.02$, $P = 0.32$), and at the section level, comparing red and white oaks ($r = 0.09$, $P = 0.11$) after controlling for the effect of pairwise geographical distances among localities (Fig. 1).

Intra-specific genetic structure

Curculio pardus and *C. occidentis* showed contrasting patterns of genetic structure. The results of the AMOVA for *C. pardus* indicate a significant genetic differentiation among populations explaining 59% of the molecular variance (d.f. = 19, $P < 0.0001$). The geographical pattern retrieved by the

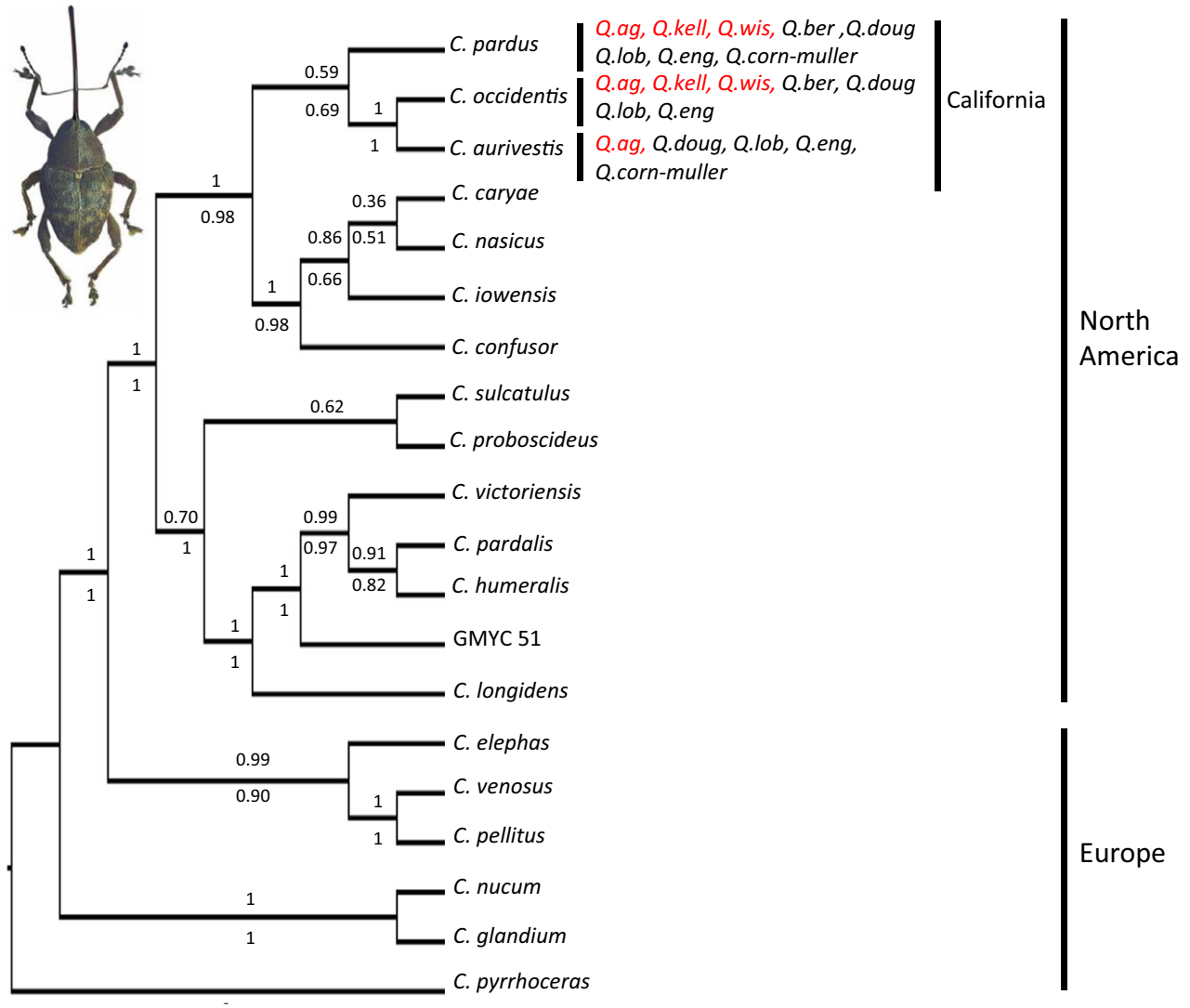

Figure 2 DNA phylogeny of two mitochondrial (cox1 and cytb) and one nuclear (EF-1a) genes for the genus *Curculio*. Tree topology was inferred using maximum likelihood (GTR+I+Gamma substitution model) and Bayesian inference. Support for each node is represented by the value of likelihood-ratio test for branch support (above the branch) and the Bayesian probability value (below the branch). Besides each weevil species is indicated the oak species (*Q. ag, Quercus agrifolia; Q. kell, Quercus kelloggii; Q. wis, Quercus wislizeni; Q. lob, Quercus lobata; Q. ber, Quercus berberidifolia; Q. doug, Quercus douglasii; Q. corn-muller, Quercus cornelius-mulleri; Q. eng, Quercus engelmannii*) in which the larvae were collected, showing also if it is a red or white oak (*Erythrobalanus* or *Leucobalanus* sections, red and black type respectively). Picture of adult *Curculio*: author R. Bonal.

SAMOVA showed three clusters (Fig. 3), explaining a 67% of the molecular variance (d.f. = 2, $P < 0.0001$). One cluster was distributed around the Central Valley from Monterrey Bay and Central Sierra Nevada northwards. The second was found on both sides of the southern half of the Valley. The third cluster grouped populations located south of the Transverse Ranges. Inter-population genetic differentiation for *C. occidentis* was lower than for *C. pardus*, accounting for 19% of the molecular variance, but still significant (d.f. = 19, $P < 0.0001$). The geographical pattern retrieved by the SAMOVA for *C. occidentis* identified just two clusters, explaining 30% of the molecular variance (d.f. = 1, $P < 0.001$). One of these clusters included all the populations around the Central Valley and the other comprised a single population south of the Transverse Ranges (Fig. 3).

Host specificity and genetic similarity

We found no evidence of intra-specific genetic differentiation among weevils according to host-oak species or sections. In the case of *C. pardus*, Mantel tests showed that genetic dissimilarity among sites was strongly correlated with geographical distance ($r = 0.46$, $P < 0.001$). However, differences in local host tree community composition had no effect on weevil intra-specific genetic differentiation either at the oak species ($r = -0.07$, $P = 0.78$) or the taxonomic section (red/

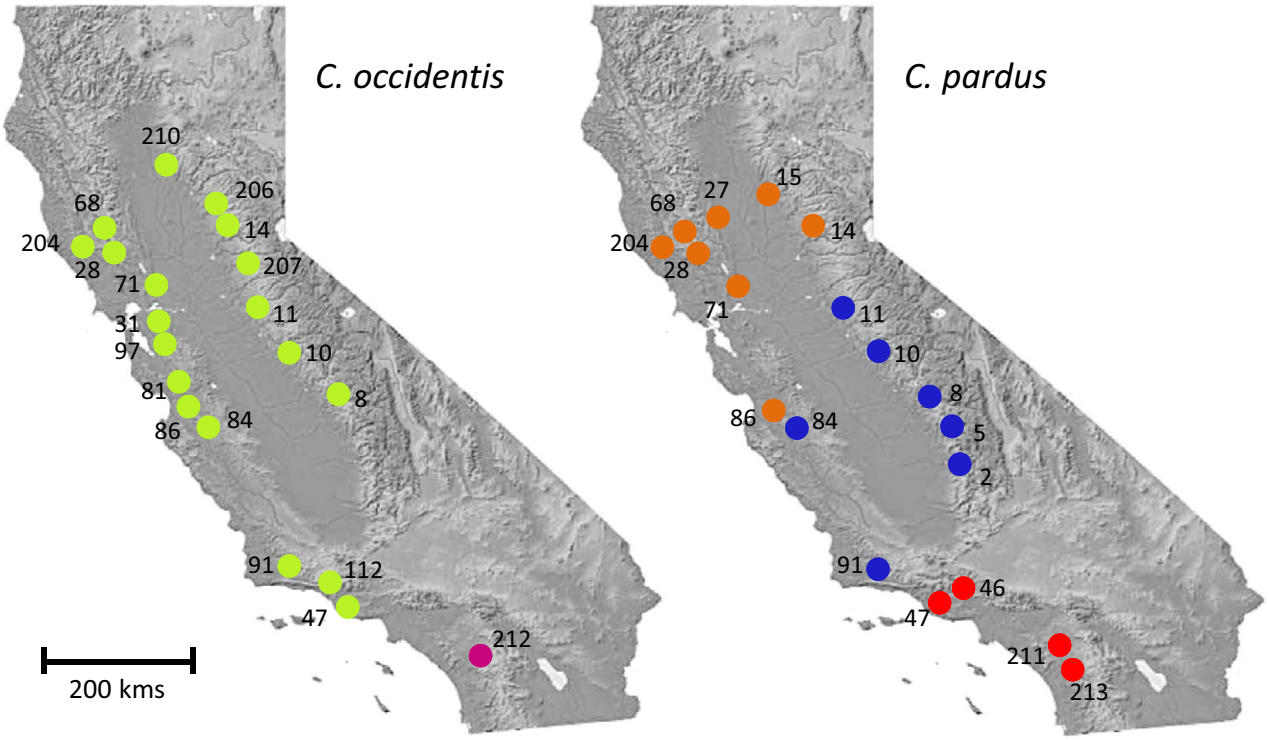

Figure 3 Maps depicting the geographical genetic structure of *Curculio occidentis* (left panel) and *C. pardus* (right panel) in California. Those localities with the same colour were included by the SAMOVA analysis within the same group. Numbers correspond to population codes described in Appendix S1.

white oaks) levels ($r = 0.01$, $P = 0.41$). Like *C. pardus*, similarity in host trees community composition among sites had no effect on *C. occidentis* genetic similarity (oak species: $r = -0.05$, $P = 0.63$; red/white oak sections: $r = -0.16$, $P = 0.11$). However, unlike *C. pardus*, pairwise geographical distance did not significantly explain genetic dissimilarity in *C. occidentis* ($r = 0.15$, $P = 0.11$).

Curculio inter-specific segregation according to acorn size

Both the size of infested acorns and the weight of the larvae of *C. pardus* and *C. occidentis* did not differ significantly. Where both weevil species co-existed, the mean size of the acorns infested by *C. pardus* and *C. occidentis* were 3.66 ± 0.30 and 3.81 ± 0.33, respectively, (paired Student's *t*-test: $t = 0.65$, d.f. = 14, $P = 0.52$). Larval weight also did not differ between the two weevil species (paired Student's *t*-test: $t = -1.81$, d.f. = 9, $P = 0.11$).

Curculio performance in the different host species

Larvae performance did not change significantly among host oaks, but did differ among localities. The weights (mean ± SE) of *C. pardus* larvae collected on *Q. agrifolia*, *Q. berberidifolia*, *Q. douglasii* and *Q. lobata* were 44 ± 4, 43 ± 3, 46 ± 1 and 49 ± 1 mg respectively. These differences among oak species were not significant ($F_{3,91} = 0.28$, $P = 0.59$), and the covariate acorn size had no significant effect either ($F_{1,91} = 0.36$, $P = 0.54$). Locality (included as a random effect) was the only significant explanatory variable ($F_{12,91} = 2.07$, $P = 0.02$). We found similar results for *C. occidentis*. Larval weights (mean ± SE) were 35 ± 1, 37 ± 2, 39 ± 2, 41 ± 1 and 42 ± 2 mg within *Q. agrifolia*, *Q. berberidifolia*, *Q. douglasii*, *Q. lobata* and *Q. wislizenii* respectively. As we saw in *C. pardus*, neither the fixed factor (oak species) ($F_{4,135} = 2.06$, $P = 0.27$) nor the covariate (acorn mass) ($F_{1,135} = 0.73$, $P = 0.39$) significantly explained *C. occidentis* larval weight. In contrast to *C. pardus*, locality (random effect) had no effect on larval weight for *C. occidentis* ($F_{17,135} = 3.98$, $P = 0.28$).

When larvae feeding on the same oak species (*Q. lobata*) were compared, there was a significant effect of the locality on larval weight of both *C. pardus* ($F_{7,55} = 2.56$, $P = 0.02$; Fig. 4a) and *C. occidentis* ($F_{12,71} = 3.87$, $P < 0.0001$; Fig. 4b), even after controlling for acorn mass, which had no significant effect ($F_{1,55} = 2.16$, $P = 0.14$; Fig. 4a for *C. pardus* and $F_{1,71} = 1.49$, $P = 0.42$; Fig. 4b for *C. occidentis*).

DISCUSSION

Our results show a strong trophic niche overlap among Californian acorn weevils. Additionally, larval performance did not differ between host species, supporting a lack of specialization. Species turnover and intra-specific genetic structure of weevils were spatially arranged independently of host-oak

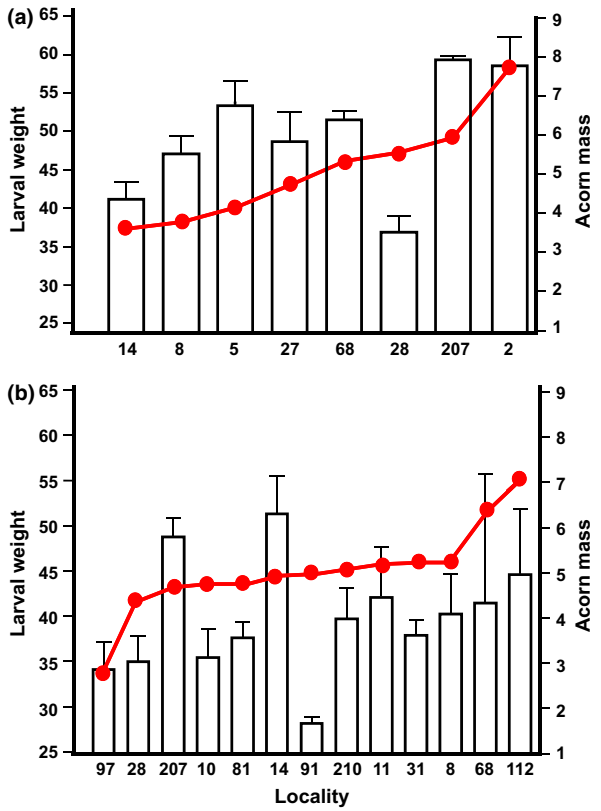

Figure 4 Bar-plots showing the mass (left *y*-axis, milligrams, mean ± SE) of (a) *Curculio pardus* and (b) *C. occidentis* larvae that developed *ad libitum* feeding on *Quercus lobata* acorns at different localities. The red dots within the bars connected with the red line are the mean mass of the acorns exploited by each *Curculio* species at each locality (right *y*-axis, grams). Localities are arranged on the *x*-axis in increasing order of mean acorn mass.

species assembly, which suggests that historical processes have contributed to the assemblage of acorn weevil communities across California.

Californian *Curculio* form a monophyletic subclade within the North American clade, probably due to historic isolation in a region with a high number endemic plants and animals (Nixon, 2002; Calsbeek *et al.*, 2003). All species we examined in California were observed feeding on both red and white oaks, indicating that strict host specificity has not triggered speciation in Californian weevils. Moreover, DNA taxonomy ruled out any cryptic speciation and trophic niche segregation among morphologically similar species. At the *Quercus* species level, the absence of *C. occidentis* within the samples collected from *Q. cornellius-mulleri* is probably a matter of sample size, as that oak was present in just one site in which few weevils were collected. Similarly, although *C. aurivestis* was not found at any site with *Q. wislizenii*, *Q. kellogii* and *Q. berberidifolia* present, it was the least common weevil species collected. This may be a question of range limitation rather than of host specificity, as when the oak species on which *C. aurivestis* had been collected at other locations shared the same location with these three oaks, this weevil species was absent.

The spatial arrangement of genetic variance across weevil populations suggests an important role of the complex geographical history of California in structuring weevil communities. The populations south of the Transverse Range for both *Curculio* species differed significantly from the rest of the distribution to the north (Fig. 3), a pattern frequently found in many Californian plant and animal taxa (Calsbeek *et al.*, 2003; Davis *et al.*, 2008; Vandergast *et al.*, 2008). We identified a genetic split between the northern and southern halves of the Central Valley within *C. pardus*, with boundaries at Monterrey Bay and Sierra Nevada. Areas with greater genetic connectivity among Sierra and coastal populations of *C. pardus* are the same valley corridors identified by the host-oak *Q. lobata* (Gugger *et al.*, 2013). Historically, the populations of many Californian species were split by the Sierra Nevada uplifts and the flooding of extensive areas of the San Joaquin Valley via the inland waterway from Monterrey Bay (*c.* 5–2.5 Ma) (e.g. Kuchta *et al.*, 2009; Satler *et al.*, 2011; Gugger *et al.*, 2013). Nevertheless, the barrier effect of the Transverse Range pre-dates this division, creating a stronger separation for numerous species (Calsbeek *et al.*, 2003; Vandergast *et al.*, 2008). If *C. occidentis* spread northwards later than *C. pardus* (when those barriers had already disappeared) less differentiation among populations of the former species north of these mountains would have established. Alternatively, previous studies have demonstrated that the dispersal abilities can differ among *Curculio* species (Govindan *et al.*, 2012; Pélisson *et al.*, 2013a,b). If the dispersal abilities of *C. occidentis* are higher than those of *C. pardus,* the above mentioned past geographical barrier might have had less effect in the former.

Our extensive sampling showed that larval weight, which is a strong proxy of fitness (Desouhant *et al.*, 2000; Bonal *et al.*, 2012), differed among localities but not among host oaks. As all larvae were grown experimentally in the same environment we could rule out direct local effects on larval growth. Hence, differences in larval weight among localities are more likely the result of random drift or local adaptation (Mousseau & Roff, 1989). These effects were more pronounced in *C. pardus*, which differed significantly among localities and when considering only the localities where *Q. lobata* was present. Given that *C. pardus* also exhibited a stronger genetic association with geography, it is possible that this difference may signal underlying genetic differences and local adaptation.

The lack of differences in larval performance between host oaks supports the absence of specificity, as specialists achieve a higher fitness on their preferred hosts (Sword *et al.*, 2005). Variation in acorn tannin content among oak species (Pyare *et al.*, 1993) might have promoted specialization. Recent studies have found mechanisms (endosymbiotic bacteria) in some *Curculio* spp. that facilitate host specific digestive ability (Toju & Fukatsu, 2011; Merville *et al.*, 2013). Nevertheless, our results do not suggest this type of adaptation in Californian acorn weevils, as larval performance did not differ among host oaks. We did not find inter-specific segregation

according to acorn size either. As body size is the common determinant of acorn size specialization (Bonal *et al.*, 2011), and it did not differ significantly among *Curculio* spp., it does not seem likely that any size segregation is occurring.

The lack of trophic niche partitioning within these acorn weevils is puzzling, but may be driven by stochastic resource availability. Similar patterns in other herbivorous arthropods have been often attributed to nutritional advantages of a generalist diet or the lower vulnerability to parasitoids (Bernays & Graham, 1988; McCormick *et al.*, 2012). Our findings in acorn weevils may be the product of an unpredictable and not always synchronized acorn crop among co-occurring oak species (Koenig *et al.*, 1994; Espelta *et al.*, 2008). When resource availability is unpredictable, a generalist weevil species would be more likely to find a suitable acorn to oviposit each year. On the contrary, a narrow specialist strategy would only persist if the increased fitness on the preferred host compensates the risks of not reproducing when that host is unavailable. For instance, leaf chewers and miners exploit a food source (i.e. leafs) that is predictably abundant each year, thus most species are frequently specialized on specific oak species or taxonomic sections (Cook *et al.*, 2002; Pearse & Hipp, 2009).

The absence of segregation among host species and acorn sizes draws a picture of weevil communities with a strong inter-specific trophic niche overlap. The Co-existence Theory (Chesson, 2000) proposes that storage effects stabilize population levels to prevent complete competitive dominance when species are affected differently by environmental variation in space and/or time (Chesson, 2000). This mechanism fits well with *Curculio* spp. life histories, as they feed on a resource (acorns) available for a limited annual time period with an unpredictable abundance due to oak mast-seeding (Koenig *et al.*, 1994; Espelta *et al.*, 2008). In turn, adult weevils emerge and reproduce after an underground diapause that may last between 1 to 4 years depending on the species. This inter-specific time partitioning across years means that unpredictable large crops do not always benefit the same species (Venner *et al.*, 2011), and allows one taxa to get largely out-competed for resources 1 year, yet still maintain a stable population. It is possible that resource partitioning across years may account for our results, however, future studies analysing long-term weevil abundance are necessary in order to verify such a pattern.

Inter-specific differences in reproductive phenology lead in some cases to an additional within year time partitioning that favours co-existence (Pélisson *et al.*, 2013a,b). In years of low acorn production, early reproducing species occupy most available acorns. On the contrary, late reproducing ones are benefited when the number of acorns is not limiting. In those years, their larvae grow within larger full sized acorns and are more likely to finish their development successfully compared to early reproducing species (Bonal *et al.*, 2011; Venner *et al.*, 2011). When there is temporal segregation within the same year, the size of the infested acorns differs among weevil species (Bonal *et al.*, 2011), and this is not what we found for Californian acorn weevils. However, as we do not have detailed information about their emergence timing, we cannot rule out that their co-existence might also be stabilized by within year time partitioning.

In conclusion, our results reveal no trophic specialization within *Curculio* species indicating the potential importance of historical processes (e.g. dispersal, extinction/migration dynamics) in the structuring of acorn weevil communities across California and show that ecologically similar seed predators can co-exist exploiting the same host species. The marked inter-annual variability and unpredictability of acorn crops in mixed oak forests may have selected against narrow specialization, and facilitated co-existence by means of an inter-specific time partitioning of the resources. This study shows the usefulness of wide geographical records of parasitic insects and their host plants to set light on the processes underlying species diversity.

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SUPPORTING INFORMATION

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

Appendix S1 Locality, geographical location and host-oak for each *Curculio* spp.

BIOSKETCH

Raul Bonal is interested in plant–animal interactions with special emphasis on seed-feeding insects. He has gradually moved from local studies (just one plant and one insect species) to large scale ones involving multiple species and incorporating phylogenetics/population genetic analyses. He is currently investigating the ecological and historical factors ruling the species assemblages of granivorous insects at different spatial scales.

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