



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

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1 **Original article**

2 **Diversity in insect seed parasite guilds at large geographical**
3 **scale: the role of host-specificity and spatial distance**

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26 **ABSTRACT**

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

35 **Location** An 800km transect across California, USA.

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

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

49 **Main conclusions** Historical processes have contributed to the structuring of
50 acorn weevil communities across California. Trophic niche overlapped among

1
2 51 species, indicating that ecologically similar species can co-exist. Acorn crop inter-
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4 52 annual variability and unpredictability in mixed oak forests may have selected
5
6 53 against narrow specialization, and facilitated co-existence by means of an inter-
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8 54 specific time partitioning of the resources. Wide scale geographical records of
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11 55 parasitic insects and their host plants are necessary to understand the processes
12
13 56 underlying species diversity.
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20 58 **Keywords** acorn, California, *Quercus* spp., seed-feeding insects, spatial
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22 59 autocorrelation, species turnover.
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75 INTRODUCTION

76 The different potential factors underlying species assemblages have been widely
77 debated but still remain a current topic in ecology and, particularly, in plant-insect
78 interactions research. The Competitive Exclusion Principle states that multiple
79 species cannot utilize the same limiting trophic resources indefinitely. Thus
80 selection on each species results from inter-specific specialization that guarantees
81 some portion of the resource is acquired (Hardin 1960). In contrast, the neutral
82 Theory of Biodiversity assumes that competing species are ecologically similar,
83 and predicts that the structure of their communities will depend on historical
84 demographic processes like extinction/migration dynamics (Bell 2001). The Co-
85 existence Theory (Chesson, 2000) supports the Neutral Theory of Biodiversity
86 proposing mechanisms to explain how co-existing competing species can
87 sustainably maintain an overlapping trophic niche.

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

95 Insect parasitism on plants is a good example of how intimate species
96 interactions and competition for limited resources can drive specialization (e.g.
97 Cook *et al.*, 2002). Many parasitic insects carry morphological, behavioural, and
98 physicochemical traits adapted to the characteristics of their host plants (i.e.
99 phenology, leaf or seed morphology, physicochemical defences) (Pearse & Hipp,

1
2 100 2009; Ygel *et al.*, 2011). Trophic specialization drives phylogenetic specificity,
3
4 101 which has a variable taxonomic spread: from taxa that feed on plants of the same
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6 102 family or genus to extreme specialists that exploit only one species (reviewed in
7
8 103 Barrett & Heil, 2012).

10 104 The degree to which specificity is possible within parasitic insects is
11
12 105 dependent on the strength of homogenizing and differentiating forces across their
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14 106 range. Specificity may start at the intra-specific level, when local adaptation to
15
16 107 different hosts drives divergence between populations of the same parasite species
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18 108 (Thompson, 1999; Drummond *et al.*, 2010). Populations separated in space, with
19
20 109 reduced gene flow homogenizing genetic variance, have a greater likelihood of
21
22 110 diverging, with taxa splitting into new species that optimize their performance on
23
24 111 the preferred hosts to increase their relative fitness (Sword *et al.*, 2005).
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26 112 Nevertheless, differentiation is not always morphologically evident, and may
27
28 113 require molecular techniques to discern species (i.e. specialized cryptic species in
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30 114 Murray *et al.*, 2007; review in Barrett & Heil, 2012). Regional scale records of
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32 115 parasitic insects and their host plants could identify the degree to which host
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34 116 specificity drives regional species diversity, while accounting for the influence of
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36 117 geographic separation and climatic variance that may additionally drive local
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38 118 adaptation.

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40 119 We chose Californian acorn weevils as a case-study because California is a
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42 120 biodiversity hotspot with physical barriers, heterogeneous habitats, and climatic
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44 121 conditions that have dramatically shaped species diversification, distribution, and
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46 122 genetic structure (Calsbeek *et al.*, 2003; Davis *et al.*, 2008). Weevils (Coleoptera:
47
48 123 Curculionidae) parasitize oak acorns worldwide (Bonal *et al.*, 2011; Toju &
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50 124 Fukatsu, 2011; Govindan *et al.*, 2012) and (like most of their endemic host oaks
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2 125 (Nixon 2002)) are widely distributed across California (Gibson 1969). With such
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4 126 an extensive distribution over a climatically and topographically diverse region,
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6 127 independent geographic effects may have played a significant role in structuring
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8 128 weevil communities. Nevertheless, previous weevil studies have sought only
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10 129 ecological explanations for species structuring. Govindan *et al.* (2012) reported
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12 130 inter-specific segregation and showed that weevils that fed on acorns of their
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14 131 preferred oak species had a greater survival likelihood. Other authors have
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16 132 hypothesized that inter-specific diversification of weevils has been driven by body
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18 133 size adaptation to the size of the acorns exploited (Hughes & Vogler, 2004a, Bonal
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20 134 *et al.* 2011). However, in all cases host records come from taxonomic oriented
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22 135 articles (Gibson 1969), or population-level studies carried out at a small spatial
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24 136 scale examining only a few of the potential host species.
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29 137 Our main objective was to test *Curculio* spp. host-specificity after
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31 138 accounting for variation in the geographic structure of the parasite species
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33 139 prevalence, genetic differentiation, and performance. Weevils were collected from
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35 140 eight different oak species from the two major sections (*Erythrobalanus* and
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37 141 *Leucobalanus*) within the genus *Quercus*. We sampled the majority of hosts and
38
39 142 parasite geographic ranges and performed DNA-based species delimitation of
40
41 143 weevils to detect potential host-specialized cryptic taxa. Specifically, i) we studied
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43 144 host species specialization in acorn weevils by assessing whether species turnover
44
45 145 and intra-specific genetic differentiation between localities depended on host
46
47 146 species similarity or simple spatial proximity; ii) we studied acorn size
48
49 147 specialization by comparing the size of the acorns exploited by the different weevil
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51 148 species within the same locality; iii) Finally, we examined weevil weight upon
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2 149 emerging from an acorn to analyse the potential impact of host-specific ability on
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4 150 weevil performance.

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8 152 **MATERIAL AND METHODS**

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12 154 *Study area and species*

13 155 From late September to mid October 2010 we sampled at a total of 29 localities
14
15 156 widespread over the state of California (North-South and East-West ranges of 805
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17 157 km and 531 km, respectively) (Appendix S1; Fig. 1). Each site was georeferenced
18
19 158 and we sampled all oak species present, when availability and spatio-temporal
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21 159 variation in crop production (Koenig *et al.*, 1994) permitted. We collected acorns
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23 160 from the most widespread oak species of California, as well as some narrowly
24
25 161 distributed endemics, including both red oaks, *Erythrobalanus* section (*Q. agrifolia*,
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27 162 *Q. kelloggii*, *Q. wislizenii*), and white oaks, *Leucobalanus* section (*Q. lobata*, *Q.*
28
29 163 *douglasii*, *Q. engelmannii*, *Q. berberidifolia*, *Q. cornellius-mulleri*) (Appendix S1).

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31 164 Weevils (*Curculio* spp. Coleoptera, Curculionidae) are the main pre-
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33 165 dispersal acorn predators and may attack more than 80% of the crop (Gibson,
34
35 166 1969; Bonal *et al.*, 2007; Espelta *et al.*, 2008). Predation occurs by parasitism,
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37 167 when *Curculio* spp. females oviposit into the acorns, where larvae feed on the
38
39 168 cotyledons as they develop. To date, three species of acorn weevils have been
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41 169 recorded in California (*C. pardus*, *C. occidentis* and *C. aurivestis*) with most of their
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43 170 populations located within the study area, spreading marginally to the North and
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45 171 East (Gibson, 1969). To confirm that they do not spread further East, we included
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47 172 in the analyses weevil larvae collected in Utah (37° 02' 45'', 112° 43' 23'').
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2 173 Adult weevils were collected by gently shaking the oak branches over a
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4 174 white blanket and larvae were collected from infested acorns. At the laboratory
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6 175 facilities of the University of California Los Angeles (UCLA) adults were identified
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8 176 to the species level following Gibson (1969). Infested acorns were separated into
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10 177 plastic dishes, and kept at 20°C to provide identical development conditions to all
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12 178 larvae. After emerging, larvae were weighed and then stored in tubes filled with
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14 179 99% ethanol for later DNA extraction.
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20 181 *DNA extraction and sequencing*

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22 182 We selected 672 weevils for molecular analyses, balancing the number of
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24 183 individuals between host oak species and localities. We included 14 adults
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26 184 representing the three species of Californian acorn *Curculio*, the rest (90% of the
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28 185 samples) were larvae to be further identified by means of DNA taxonomy (see
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30 186 Pinzon-Navarro *et al.*, 2010 for a similar procedure). DNA was extracted from
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32 187 insect tissue according to the Aljanabi & Martínez (1997) salt extraction protocol.
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35 188 Individuals were genotyped by amplifying two mitochondrial genes,
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37 189 cytochrome oxidase I (*cox1*) and cytochrome B (*cytb*). We used the primers C1-J-
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39 190 2183 (Jerry) and L2- N-3014 (Pat) for the first and the universal primers CB1 and
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41 191 CB2 for the second. In addition, we amplified a fragment of the nuclear gene
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43 192 encoding elongation factor 1 α (EF-1 α) using EF1-R and EF1-F primers (see
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45 193 Hughes & Vogler, 2004b for details on the PCR conditions for the three genes).
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47 194 Sequence chromatograms were assembled and edited using Sequencher 4.6 (Gene
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49 195 Codes Corp., Ann Arbor, MI, USA). The sequences of the three genes (*cox1*, *cytb* and
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51 196 EF-1 α) were trimmed to 711, 413 and 581 base pairs respectively to reduce the
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197 proportion of missing data. In the case of the nuclear gene EF-1 α some sequences
198 contained gaps in the intron region.

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200 *Species delimitation and phylogenetic analyses*

201 We pooled the *cox1* sequences of all individuals to delimit the different species
202 according to the generalized mixed Yule-coalescent (GMYC) model (Pons *et al.*,
203 2006) implemented in R package 'splits', in which we used the 'single threshold'
204 option (Pons *et al.*, 2006). We built a Maximum Likelihood (ML) tree including one
205 copy of each haplotype applying a GTR + I + Gamma substitution model –according
206 to the results of jModelTest 0.1.1 (Posada, 2008). The gall feeding weevil *C.*
207 *pyrrhoceras* was used as outgroup, as it presents the greatest divergence to the
208 other *Curculio* species for the three genes analysed (see Hughes & Vogler, 2004b).

209 The analysis was performed with RAxML 7.0.4 (Stamatakis, 2006) and the
210 resulting tree was made ultrametric under a molecular clock model in
211 PAUP*4.0b10 (Swofford, 2002) with the parameters estimated from the ML
212 search. The GMYC model tracks the tree branching rates and detects the transition
213 from among-species to within-population branching patterns, delimiting
214 'independently evolving' mtDNA clusters. These clusters are called GMYC
215 (putative) species and, if they include sequences from known Linnean species, they
216 may serve to differentiate otherwise indistinguishable specimens like weevil
217 larvae (for which there are no morphological keys) (Pinzon-Navarro *et al.*, 2010).

218 One individual per GMYC group was chosen for a more detailed phylogenetic
219 analysis based on three genes (*cox1*, *cytb* and EF-1 α). Our main objective was to
220 assess the phylogenetic relationships among the Californian weevils to investigate
221 whether host-shifts may be involved in species splitting. To do so we pooled the

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2 222 Californian sequences with those of another 17 species of American and European
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4 223 *Curculio* (Hughes & Vogler, 2004a). The three genes were aligned separately with
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6 224 Clustal W (Thompson *et al.*, 1994). In the case of the nuclear EF-1 α we used the
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8 225 gap opening and gap extension penalties provided by default by Clustal W (15 and
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10 226 6.66, respectively), and visual inspection of the alignment showed that those
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12 227 values were accurate. Next, all genes were concatenated, realigned and our final
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14 228 sequence data file was visually revised to make sure that there were no errors. The
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16 229 gall eating *C. pyrrhoceras* was the outgroup in all phylogenies (see Hughes &
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18 230 Vogler, 2004b).

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21 231 We searched for the most reliable tree topology and calculated the support
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23 232 of the tree nodes following two methods (Maximum Likelihood and Bayesian
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25 233 Inference); comparing if the two model-based approaches yielded similar
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27 234 phylogenies. We calculated the best-fit models of nucleotide substitution for each
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29 235 of the three genes according to the Akaike Information Criterion (AIC) using
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31 236 jModelTest 0.1.1 (Posada, 2008). Maximum Likelihood analyses were performed in
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33 237 RAxML 7.2.6 (Stamatakis, 2006) and PHYML 3.0 (Guindon & Gascuel, 2006). In
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35 238 RAxML three partitions were set (one for each gene) and 10 independent searches
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37 239 conducted. PHYML was additionally used to assess the repeatability of the
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39 240 topology and also because it allows calculating the approximate Likelihood-Ratio
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41 241 Test for branch support, which is a good alternative to nonparametric bootstrap
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43 242 (Guindon & Gascuel, 2006). Bayesian inference analyses were performed with Mr
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45 243 Bayes 3.2 (Ronquist *et al.*, 2012). We used the same partitions as we used in the
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47 244 Maximum Likelihood tree (RAxML), applying a nucleotide substitution model
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49 245 specific to each gene. Two parallel runs of 2 million generations each were
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51 246 conducted using one cold and two incrementally heated Markov chains ($\Lambda=0.2$),
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2 247 sampling every 1,000 steps. We first checked one of the standard convergence
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4 248 diagnostics implemented in MrBayes and then assessed the average standard
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6 249 deviation of the split frequencies to deduce that the Markov chain had reached
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8 250 stationarity. After 500,000 generations, the average standard deviation of the split
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10 251 frequencies stabilized in values close to zero (0.001). Hence, phylogenetic trees
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12 252 were summarized using the all-compatible consensus command with 25% burn-in.
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17 254 *Intra-specific genetic structure*

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19 255 We analysed inter-population genetic differentiation in those species (*C. pardus*
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21 256 and *C. occidentis*) that had a sufficient number of specimens per sampling locality
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23 257 (see below the choice criteria). We performed analyses of the molecular variance
24
25 258 (AMOVAs) using ARLEQUIN software (Excoffier *et al.*, 2005) and also tested
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27 259 whether there was any geographic pattern in the population genetic structure
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29 260 using SAMOVA version 1.0 (Dupanloup *et al.*, 2002). This method identifies the
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31 261 optimal grouping option (K) that maximises the among-group component (FCT) of
32
33 262 the overall genetic variance. We defined the number of populations (K) and ran
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35 263 100 simulated annealing processes. We simulated different numbers of
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37 264 populations, ranging from K = 2 to K = 19, to determine the best population
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39 265 clustering option.
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43 267 *Curculio intra-specific genetic dissimilarities among hosts and localities*

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45 268 We performed intra-specific analyses on *C. pardus* and *C. occidentis* (*C. aurivestis*
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47 269 samples did not reach a sufficient number per site). We included only those
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49 270 localities in which there were sequences for at least 4 individuals per species (see
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51 271 Papadopoulou *et al.*, 2011 for a similar approach). Above this threshold we
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2 272 confirmed that there was no effect of sample size on either genetic (*C. pardus*: $r =$
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4 273 $0.31, p = 0.17, n = 20$; *C. occidentis*: $r = 0.09, p = 0.68, n = 19$) or nucleotide diversity
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6 274 (*C. pardus*: $r = 0.21, p = 0.36, n = 20$; *C. occidentis*: $r = 0.001, p = 0.99, n = 19$). We
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8 275 used Arlequin 3.1 (Excoffier *et al.*, 2005) to compute genetic dissimilarities by
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10 276 assessing the raw average number of differences among populations (Nei's D) in
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12 277 the mitochondrial gene *cox1*. DNA microsatellites (nuclear DNA) have yet to be
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14 278 developed for these species and, although they can provide finer resolution in
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16 279 genetic analysis, in other *Curculio* spp. mitochondrial markers have detected
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18 280 population structure at scales of just a few kilometres and distinguished host-
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20 281 adapted morphotypes (Toju & Sota, 2006; Toju *et al.*, 2011). Host-oak
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22 282 dissimilarities were calculated using Bray-Curtis index on the number of *Curculio*
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24 283 individuals sampled on each oak species and its correlation with intra-specific
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26 284 genetic dissimilarities was analysed controlling for the effect of the Euclidean
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28 285 geographical distance between localities using partial Mantel tests as implemented
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30 286 in the R package 'ecodist' (Goslee & Urban, 2007).
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288 *Local oak community composition and Curculio species turnover among sites*

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39 289 Due to variable insect availability it was not always possible to balance weevil
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41 290 sample size across sites and *Quercus* species, hence we included those 25 localities
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43 291 with 9 or more individuals (Appendix S1). Above that number we found no
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45 292 significant effect of sample size on either the number of species (Spearman
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47 293 correlation: $r = 0.34, p = 0.14, n = 25$) or species α -diversity (Spearman correlation:
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49 294 $r = 0.10, p = 0.60, n = 25$) collected at a site. Further, in 16 localities in which
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51 295 sample size was greater than 18, we calculated the mean rarified number of
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53 296 species standardized first for 9 and then for 18 individuals, and found no
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2 297 significant differences between the two estimates (ANOVA: $F_{1, 30} = 0.57$; $p = 0.45$).
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4 298 Species diversity and richness measures were calculated using the R package
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6 299 'vegan' (Oksanen *et al.*, 2012). Statistical analyses were performed using R (R
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8 300 Development Core Team, 2012).

10 301 We examined the influence of host-oak communities on weevil species
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12 302 compositional dissimilarity. Pairwise *Curculio* species turnover among localities
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14 303 was assessed with the Bray-Curtis index. This index is calculated using species
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16 304 presence/absence and relative abundance, making it less affected by low species
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18 305 numbers. We measured the correlation between *Curculio* spp. and host-oak
19
20 306 similarities with a partial Mantel test (10000 permutations) using the Euclidean
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22 307 geographical distance among localities as a control for potential spatial auto-
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24 308 correlation effects (Koenig, 1999). We ran this analysis using the R package
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26 309 'ecodist' (Goslee & Urban, 2007).

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31 311 *Curculio* inter-specific segregation according to host size

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34 312 We assessed whether acorns were partitioned by size among the larvae of *C.*
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36 313 *pardus* and *C. occidentis*. *Curculio aurivestis* was not included due to low sample
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38 314 sizes. The raw weight of infested acorns is an unreliable estimate of acorn size, as
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40 315 weight varies with the amount of cotyledon eaten by the larvae inside. Instead, we
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42 316 used linear dimensions of each acorn (length and width to the nearest 0.01 mm) to
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44 317 estimate acorn mass using the formula detailed in Bonal *et al.* (2007). In those
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46 318 localities where both weevil species co-existed and at least three larvae of each
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48 319 species were collected, we compared the size of the acorns exploited by each with
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50 320 a paired Student's t-test. As body size affects the size of the acorns used (Bonal *et*
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52 321 *al.*, 2011), we also compared *C. pardus* and *C. occidentis* larval weight with a paired
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2 322 Student's t-test.

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6 324 *Curculio* performance according to host species identity and host seed size

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8 325 We estimated *Curculio* performance by recording the larval weight (to the nearest

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10 326 0.1 mg) when they emerged from infested acorns. Larval weight is a key life-

11
12 327 history trait in most insects and a good fitness proxy. Within *Curculio* weevils

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14 328 larval weight determines to a large extent survival likelihood and potential

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16 329 fecundity (Desouhant *et al.*, 2000; Bonal *et al.*, 2012). We dried all the infested

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18 330 acorns at 80°C for 48 hours before opening them one month after the last larva

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20 331 had emerged. We found that the cotyledons were never depleted within our

21
22 332 samples, so any difference in larval weight would be due to the nutritional

23
24 333 quality of the acorn rather than to food constraints.

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26 334 We used an ANCOVA to test the effect of the host oak species (fixed factor)

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28 335 and acorn mass (covariate) on larval weight (dependent variable). Sampling

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30 336 locality was included as a random effect because insect body size has been shown

31
32 337 to be susceptible to changes at geographic scale due to environmental differences

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34 338 among localities (Mousseau & Roff, 1989). We ran this analysis first examining all

35
36 339 weevils, and then used just those collected on the most commonly sampled oak

37
38 340 species (*Q. lobata*) to remove any potential confounding effect of host species

39
40 341 identity. Statistical analyses were performed with Statistica 7.0 (Statsoft, Inc Tulsa,

41
42 342 OK, USA).

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348 **RESULTS**

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350 *DNA-based weevil species delimitation and phylogenetic analyses*

351 A total of 540 *cox1* sequences from adult weevils and larvae had the necessary
352 length to be included in the analyses (of these 529 were collected in California and
353 11 in Utah). We did not get sequences for the remaining 132 individuals (20%),
354 either due to PCR issues, or because the sequences obtained were not long enough.
355 These 540 sequences were collapsed into 138 different haplotypes that were used
356 to build the ultrametric clock-constrained Maximum Likelihood phylogeny
357 subjected to the GMYC analysis, which grouped the sequences in 4 clusters
358 corresponding to distinct putative species. Three of these clusters included
359 sequences obtained from both adults and larvae collected in California. All clusters
360 corresponded to just one previously-named species (*C. pardus*, *C. aurivestis* or *C.*
361 *occidentis*). All adult species assignments based on morphological characters
362 matched the species assignment based on the GMYC cluster, confirming the
363 reliability of our genetic methods and ability to accurately determine all larvae to
364 the species level. The fourth cluster corresponded to the weevil larvae collected in
365 Utah and could not be identified because their sequences did not group with any
366 acorn *Curculio* species available in GenBank; they were named GMYC 51 (Fig. 2).

367 The three phylogenies built on the combined three genes set (mitochondrial
368 *cox1* and *citb*; nuclear *EF-1 α*) retrieved the same topology (Fig. 2). The tree shows
369 a clear division between North American and European species, which form
370 different clades with a very strong branch support. The Californian acorn weevil
371 species constitute an independent subclade within the American clade (Fig. 2).
372 There is strong support for a sister species relationship between *C. aurivestis* and

1
2 373 *C. occidentis*, and comparatively low support for a monophyletic clade containing *C.*
3
4 374 *pardus*, *C. aurivestis* and *C. occidentis*, indicating that the relationship of *C. pardus* to
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6 375 the other two species is less certain.
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10 377 *Host-specificity and species turnover*

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12 378 The phylogenetic tree shows that host shifts between oak species or sections (red
13
14 379 and white oaks) were not involved in the speciation of the Californian acorn
15
16 380 *Curculio* spp. (Fig. 2). *Curculio pardus* and *C. occidentis* were present on all *Quercus*
17
18 381 spp. sampled with the exception of *C. occidentis* on *Q. cornellius-mulleri*. The scarce
19
20 382 *C. aurivestis* was not found on *Q. wislizenii*, *Q. kelloggii* and *Q. berberidifolia*.
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24 383 Species distribution patterns were defined by geographic restriction and
25
26 384 not host tree assembly (Fig. 1). The Mantel test showed that geographically more
27
28 385 distant populations harboured more dissimilar *Curculio* spp. communities ($r =$
29
30 386 0.14 , $p = 0.03$), but host oak similarity among localities was non-significant when
31
32 387 examined at the species ($r = 0.02$, $p = 0.32$), and at the section level, comparing red
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34 388 and white oaks ($r = 0.09$, $p = 0.11$) after controlling for the effect of pairwise
35
36 389 geographical distances among localities (Fig. 1).
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41 391 *Intra-specific genetic structure*

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43 392 *Curculio pardus* and *C. occidentis* showed contrasting patterns of genetic structure.
44
45 393 The results of the AMOVA for *C. pardus* indicate a significant genetic differentiation
46
47 394 among populations explaining 59% of the molecular variance ($df = 19$, $p < 0.0001$).
48
49 395 The geographical pattern retrieved by the SAMOVA showed three clusters (Fig. 3),
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51 396 explaining a 67% of the molecular variance ($df = 2$, $p < 0.0001$). One cluster was
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53 397 distributed around the Central Valley from Monterrey Bay and Central Sierra
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2 398 Nevada northwards. The second was found on both sides of the southern half of
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4 399 the Valley. The third cluster grouped populations located south of the Transverse
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6 400 Ranges. Inter-population genetic differentiation for *C. occidentis* was lower than for
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8 401 *C. pardus*, accounting for 19% of the molecular variance, but still significant (df
9
10 402 =19, $p < 0.0001$). The geographical pattern retrieved by the SAMOVA for *C.*
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12 403 *occidentis* identified just two clusters, explaining 30% of the molecular variance (df
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14 404 = 1, $p < 0.001$). One of these clusters included all the populations around the
15
16 405 Central Valley and the other comprised a single population south of the Transverse
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18 406 Ranges (Fig. 3).
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24 408 *Host-specificity and genetic similarity*

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26 409 We found no evidence of intra-specific genetic differentiation among weevils
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28 410 according to host oak species or sections. In the case of *C. pardus*, Mantel tests
29
30 411 showed that genetic dissimilarity among sites was strongly correlated with
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32 412 geographic distance ($r = 0.46$, $p < 0.001$). However, differences in local host tree
33
34 413 community composition had no effect on weevil intra-specific genetic
35
36 414 differentiation either at the oak species ($r = -0.07$, $p = 0.78$) or the taxonomic
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38 415 section (red/white oaks) levels ($r = 0.01$, $p=0.41$). Like *C. pardus*, similarity in host
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40 416 trees community composition among sites had no effect on *C. occidentis* genetic
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42 417 similarity (oak species: $r = -0.05$, $p = 0.63$; red/white oak sections: $r = -0.16$, $p =$
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44 418 0.11). However, unlike *C. pardus*, pairwise geographical distance did not
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46 419 significantly explain genetic dissimilarity in *C. occidentis* ($r = 0.15$, $p=0.11$).
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53 421 *Curculio inter-specific segregation according to acorn size*
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2 422 Both the size of infested acorns and the weight of the larvae of *C. pardus* and *C.*
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4 423 *occidentis* did not differ significantly. Where both weevil species co-existed, the
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6 424 mean size of the acorns infested by *C. pardus* and *C. occidentis* were 3.66 ± 0.30 and
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8 425 3.81 ± 0.33 respectively (paired Student's *t*-test: $t = 0.65$, $df = 14$, $p = 0.52$). Larval
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10 426 weight also did not differ between the two weevil species (paired Student's *t*-test: t
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12 427 $= -1.81$, $df = 9$, $p = 0.11$).

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16 17 429 *Curculio* performance in the different host species

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19 430 Larvae performance did not change significantly among host oaks, but did differ
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21 431 among localities. The weights (mean \pm SE) of *C. pardus* larvae collected on *Q.*
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23 432 *agrifolia*, *Q. berberidifolia*, *Q. douglasii* and *Q. lobata* were 44 ± 4 , 43 ± 3 , 46 ± 1 and
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25 433 49 ± 1 milligrams, respectively. These differences among oak species were not
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27 434 significant ($F_{3, 91} = 0.28$, $p = 0.59$), and the covariate acorn size had no significant
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29 435 effect either ($F_{1, 91} = 0.36$, $p = 0.54$). Locality (included as a random effect) was the
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31 436 only significant explanatory variable ($F_{12, 91} = 2.07$, $p = 0.02$). We found similar
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33 437 results for *C. occidentis*. Larval weights (mean \pm SE) were 35 ± 1 , 37 ± 2 , 39 ± 2 , 41 ± 1
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35 438 and 42 ± 2 milligrams within *Q. agrifolia*, *Q. berberidifolia*, *Q. douglasii*, *Q. lobata*, and
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37 439 *Q. wislizenii*, respectively. As we saw in *C. pardus*, neither the fixed factor (oak
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39 440 species) ($F_{4, 135} = 2.06$, $p = 0.27$) nor the covariate (acorn mass) ($F_{1, 135} = 0.73$, $p =$
40
41 441 0.39) significantly explained *C. occidentis* larval weight. In contrast to *C. pardus*,
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43 442 locality (random effect) had no effect on larval weight for *C. occidentis* ($F_{17, 135} =$
44
45 443 3.98 , $p = 0.28$).

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47 444 When larvae feeding on the same oak species (*Q. lobata*) were compared,
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49 445 there was a significant effect of the locality on larval weight of both *C. pardus* ($F_{7, 55}$
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51 446 $= 2.56$, $p = 0.02$; Fig. 4a) and *C. occidentis* ($F_{12, 71} = 3.87$, $p < 0.0001$; Fig. 4b), even
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2 447 after controlling for acorn mass, which had no significant effect ($F_{1,55} = 2.16$, $p =$
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4 448 0.14; Fig. 4a for *C. pardus*, and $F_{1,71} = 1.49$, $p = 0.42$; Fig. 4b for *C. occidentis*).

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8 450 **DISCUSSION**

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10 451 Our results show a strong trophic niche overlap among Californian acorn weevils.

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12 452 Additionally, larval performance did not differ between host species, supporting a

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14 453 lack of specialization. Species turnover and intra-specific genetic structure of

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16 454 weevils were spatially arranged independently of host oak species assembly,

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18 455 which suggests that historical processes have contributed to the assemblage of

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20 456 acorn weevil communities across California.

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22 457 Californian *Curculio* form a monophyletic subclade within the North

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24 458 American clade, probably due to historic isolation in a region with a high number

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26 459 endemic plants and animals (Nixon, 2002; Calsbeek *et al.*, 2003). All species we

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28 460 examined in California were observed feeding on both red and white oaks,

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30 461 indicating that strict host-specificity has not triggered speciation in Californian

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32 462 weevils. Moreover, DNA taxonomy ruled out any cryptic speciation and trophic

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34 463 niche segregation among morphologically similar species. At the *Quercus* species

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36 464 level, the absence of *C. occidentis* within the samples collected from *Q. cornellius-*

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38 465 *mulleri* is probably a matter of sample size, as that oak was present in just one site

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40 466 in which few weevils were collected. Similarly, although *C. aurivestis* was not found

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42 467 at any site with *Q. wislizenii*, *Q. kellogii* and *Q. berberidifolia* present, it was the least

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44 468 common weevil species collected. This may be a question of range limitation rather

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46 469 than of host-specificity, as when the oak species on which *C. aurivestis* had been

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48 470 collected at other locations shared the same location with these three oaks, this

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50 471 weevil species was absent.

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2 472 The spatial arrangement of genetic variance across weevil populations
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4 473 suggests an important role of the complex geographic history of California in
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6 474 structuring weevil communities. The populations south of the Transverse Range
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8 475 for both *Curculio* species differed significantly from the rest of the distribution to
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10 476 the north (Fig. 3), a pattern frequently found in many Californian plant and animal
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12 477 taxa (Calsbeek *et al.*, 2003; Davis *et al.*, 2008; Vandergast *et al.*, 2008). We
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14 478 identified a genetic split between the northern and southern halves of the Central
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16 479 Valley within *C. pardus*, with boundaries at Monterrey Bay and Sierra Nevada.
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18 480 Areas with greater genetic connectivity among Sierra and coastal populations of *C.*
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20 481 *pardus* are the same valley corridors identified by the host oak *Q. lobata* (Gugger *et*
21
22 482 *al.*, 2013). Historically, the populations of many Californian species were split by
23
24 483 the Sierra Nevada uplifts and the flooding of extensive areas of the San Joaquin
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26 484 Valley via the inland waterway from Monterrey Bay (ca. 5 to 2.5 million years ago)
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28 485 (e.g. Kuchta *et al.*, 2009; Satler *et al.*, 2011; Gugger *et al.*, 2013). Nevertheless, the
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30 486 barrier effect of the Transverse Range predates this division, creating a stronger
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32 487 separation for numerous species (Calsbeek *et al.*, 2003; Vandergast *et al.*, 2008). If
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34 488 *C. occidentis* spread northwards later than *C. pardus* (when those barriers had
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36 489 already disappeared) less differentiation among populations of the former species
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38 490 north of these mountains would have established. Alternatively, previous studies
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40 491 have demonstrated that the dispersal abilities can differ among *Curculio* species
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42 492 (Govindan *et al.*, 2012; Péliesson *et al.*, 2013). If the dispersal abilities of *C.*
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44 493 *occidentis* are higher than those of *C. pardus*, the above mentioned past
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46 494 geographical barrier might have had less effect in the former.

495 Our extensive sampling showed that larval weight, which is a strong proxy
496 of fitness (Desouhant *et al.*, 2000, Bonal *et al.*, 2012), differed among localities but

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2 497 not among host oaks. As all larvae were grown experimentally in the same
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4 498 environment we could rule out direct local effects on larval growth. Hence,
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6 499 differences in larval weight among localities are more likely the result of random
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8 500 drift or local adaptation (Mousseau & Roff, 1989). These effects were more
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10 501 pronounced in *C. pardus*, which differed significantly among localities and when
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12 502 considering only the localities where *Q. lobata* was present. Given that *C. pardus*
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14 503 also exhibited a stronger genetic association with geography, it is possible that this
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16 504 difference may signal underlying genetic differences and local adaptation.
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20 505 The lack of differences in larval performance between host oaks supports
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22 506 the absence of specificity, as specialists achieve a higher fitness on their preferred
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24 507 hosts (Sword *et al.*, 2005). Variation in acorn tannin content among oak species
25
26 508 (Pyare *et al.*, 1993) might have promoted specialization. Recent studies have found
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28 509 mechanisms (endosymbiotic bacteria) in some *Curculio* spp. that facilitate host
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30 510 specific digestive ability (Toju & Fukatsu, 2011; Merville *et al.*, 2013).
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33 511 Nevertheless, our results do not suggest this type of adaptation in Californian
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35 512 acorn weevils, as larval performance did not differ among host oaks. We did not
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37 513 find inter-specific segregation according to acorn size either. As body size is the
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39 514 common determinant of acorn size specialization (Bonal *et al.*, 2011), and it did not
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41 515 differ significantly among *Curculio* spp., it does not seem likely that any size
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43 516 segregation is occurring.
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46
47 517 The lack of trophic niche partitioning within these acorn weevils is puzzling,
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49 518 but may be driven by stochastic resource availability. Similar patterns in other
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51 519 herbivorous arthropods have been often attributed to nutritional advantages of a
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53 520 generalist diet or the lower vulnerability to parasitoids (Bernays & Graham, 1988;
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55 521 McCormick *et al.*, 2012). Our findings in acorn weevils may be the product of an
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2 522 unpredictable and not always synchronized acorn crop among co-occurring oak
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4 523 species (Koenig *et al.*, 1994; Espelta *et al.*, 2008). When resource availability is
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6 524 unpredictable, a generalist weevil species would be more likely to find a suitable
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8 525 acorn to oviposit each year. On the contrary, a narrow specialist strategy would
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10 526 only persist if the increased fitness on the preferred host compensates the risks of
11
12 527 not reproducing when that host is unavailable. For instance, leaf chewers and
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14 528 miners exploit a food source (i.e. leaf) that is predictably abundant each year, thus
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16 529 most species are frequently specialized on specific oak species or taxonomic
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18 530 sections (Cook *et al.*, 2002; Pearse & Hipp, 2009).

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20 531 The absence of segregation among host species and acorn sizes draws a
21
22 532 picture of weevil communities with a strong inter-specific trophic niche overlap.
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24 533 The Co-existence Theory (Chesson, 2000) proposes that storage effects stabilize
25
26 534 population levels to prevent complete competitive dominance when species are
27
28 535 affected differently by environmental variation in space and/or time (Chesson,
29
30 536 2000). This mechanism fits well with *Curculio* spp. life-histories, as they feed on a
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32 537 resource (acorns) available for a limited annual time period with an unpredictable
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34 538 abundance due to oak mast-seeding (Koenig *et al.*, 1994; Espelta *et al.*, 2008). In
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36 539 turn, adult weevils emerge and reproduce after an underground diapause that may
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38 540 last between 1 to 4 years depending on the species. This inter-specific time
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40 541 partitioning across years means that unpredictable large crops do not always
41
42 542 benefit the same species (Venner *et al.*, 2011), and allows one taxa to get largely
43
44 543 out competed for resources one year, yet still maintain a stable population. It is
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46 544 possible that resource partitioning across years may account for our results,
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48 545 however, future studies analysing long term weevil abundance are necessary in
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50 546 order to verify such a pattern.
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2 547 Inter-specific differences in reproductive phenology lead in some cases to
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4 548 an additional within year time partitioning that favours co-existence (Pélisson *et*
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6 549 *al.*, 2013). In years of low acorn production, early reproducing species occupy most
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8 550 available acorns. On the contrary, late reproducing ones are benefited when the
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10 551 number of acorns is not limiting. In those years, their larvae grow within larger full
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12 552 sized acorns and are more likely to finish their development successfully
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14 553 compared to early reproducing species (Bonal *et al.*, 2011, Venner *et al.*, 2011).
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16 554 When there is temporal segregation within the same year, the size of the infested
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18 555 acorns differs among weevil species (Bonal *et al.*, 2011), and this is not what we
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20 556 found for Californian acorn weevils. However, as we do not have detailed
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22 557 information about their emergence timing, we cannot rule out that their co-
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24 558 existence might also be stabilized by within year time partitioning.
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29 559 In conclusion, our results reveal no trophic specialisation within Curculio
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31 560 species indicating the potential importance of historical processes (e.g. dispersal,
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33 561 extinction/migration dynamics) in the structuring of acorn weevil communities
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35 562 across California and show that ecologically similar seed predators can co-exist
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37 563 exploiting the same host species. The marked inter-annual variability and
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39 564 unpredictability of acorn crops in mixed oak forests may have selected against
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41 565 narrow specialization, and facilitated co-existence by means of an inter-specific
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43 566 time partitioning of the resources. The present study shows the usefulness of wide
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45 567 geographical records of parasitic insects and their host plants to set light on the
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47 568 processes underlying species diversity.
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49 **SUPPORTING INFORMATION**

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52 737 Additional Supporting Information may be found in the online version of this
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54 738 article:
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2 739 **Appendix S1** Locality code, geographical location, host oak species and number of
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4 740 collected individuals for each species of Curculio in California, USA.
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10 742 **BIOSKETCH**
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14 743 Raul Bonal is interested in plant-animal interactions with special emphasis on seed
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16 744 feeding insects. He has gradually moved from local studies (just one plant and one
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18 745 insect species) to large scale ones involving multiple species and incorporating
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20 746 phylogenetics/population genetic analyses. He is currently investigating the
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22 747 ecological and historical factors ruling the species assemblages of granivorous
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24 748 insects at different spatial scales.
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30 750 Author contributions: RB, JME and VLS conceived the experiment; RB, JME, AM, JO,
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32 751 JMA and KG performed the experiments; RB, JME and JO analyzed the data; RB and
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34 752 JME wrote the manuscript; AM, KG, and VLS provided editorial advice.
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2 756 **Figure Legends**
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4 757 **Figure 1** Map of California with the locations of the 25 sampling sites where at
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6 758 least 9 weevils were sampled. The proportions of each species (*Curculio pardus*, *C.*
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8 759 *occidentis* and *C. aurivestis*) at each site are shown. Numbers correspond to
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10 760 population codes described in Appendix S1.
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15 762 **Figure 2** DNA phylogeny of two mitochondrial (cox1 and cyt b) and one nuclear
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17 763 (EF-1a) genes for the genus *Curculio*. Tree topology was inferred using Maximum
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19 764 Likelihood (GTR + I + Gamma substitution model) and Bayesian Inference. Support
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21 765 for each node is represented by the value of Likelihood-Ratio Test for branch
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23 766 support (above the branch) and the Bayesian probability value (below the branch).
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25 767 Besides each weevil species is indicated the oak species in which the larvae were
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27 768 collected, showing also if it is a red or white oak (*Erythrobalanus* or *Leucobalanus*
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29 769 sections, red and black type, respectively). Picture of adult *Curculio*: author R.
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31 770 Bonal.
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38 772 **Figure 3** Maps depicting the geographical genetic structure of *Curculio occidentis*
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40 773 (left panel) and *C. pardus* (right panel) in California. Those localities with the same
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42 774 colour were included by the SAMOVA analysis within the same group. Numbers
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44 775 correspond to population codes described in Appendix S1.
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49 777 **Figure 4** Bar-plots showing the mass (left y-axis, milligrams, mean±SE) of (a)
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51 778 *Curculio pardus* and (b) *C. occidentis* larvae that developed *ad libitum* feeding on
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53 779 *Quercus lobata* acorns at different localities. The red dots within the bars
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55 780 connected with the red line are the mean mass of the acorns exploited by each
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2 781 *Curculio* species at each locality (right y-axis, grams). Localities are arranged on
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4 782 the x-axis in increasing order of mean acorn mass.
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For Peer Review

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Figure 1

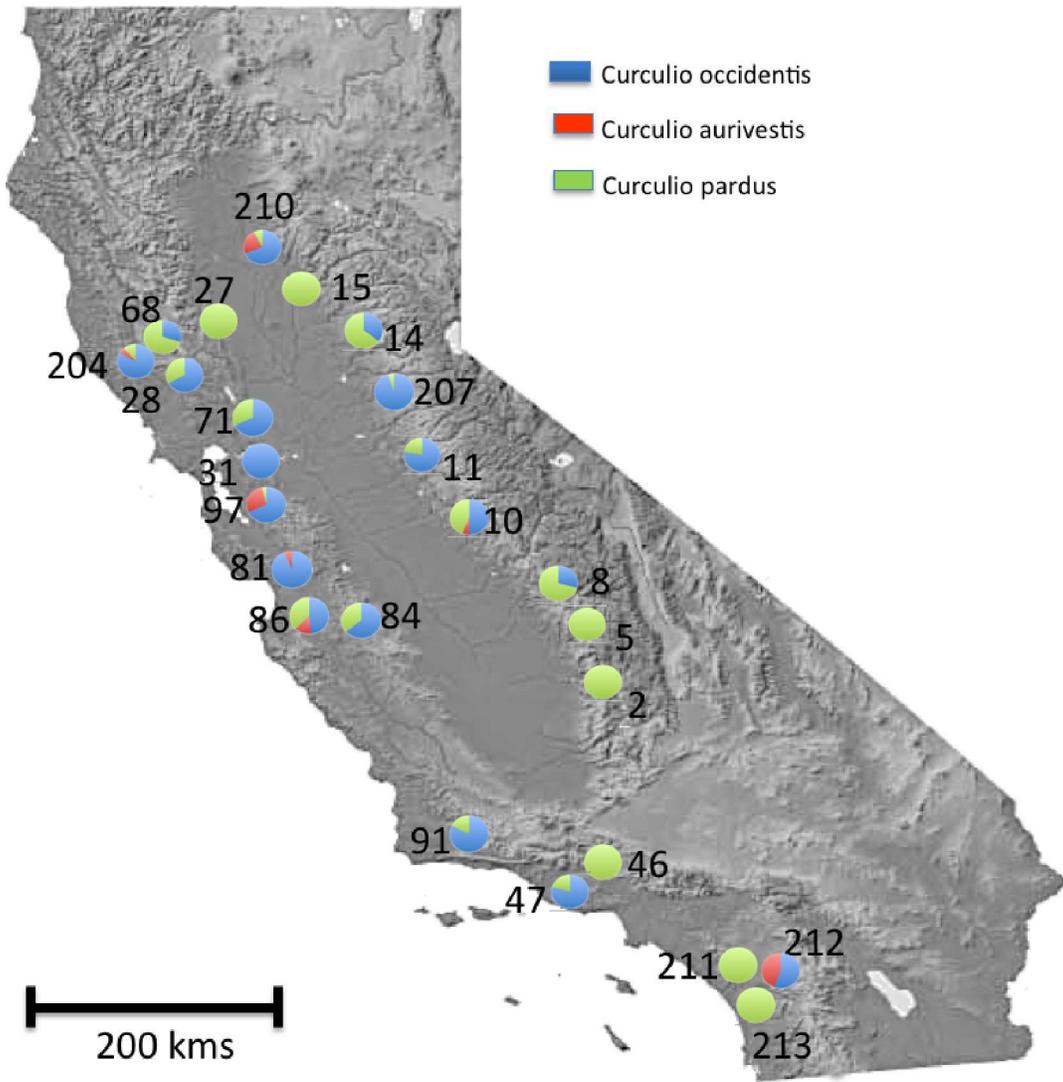
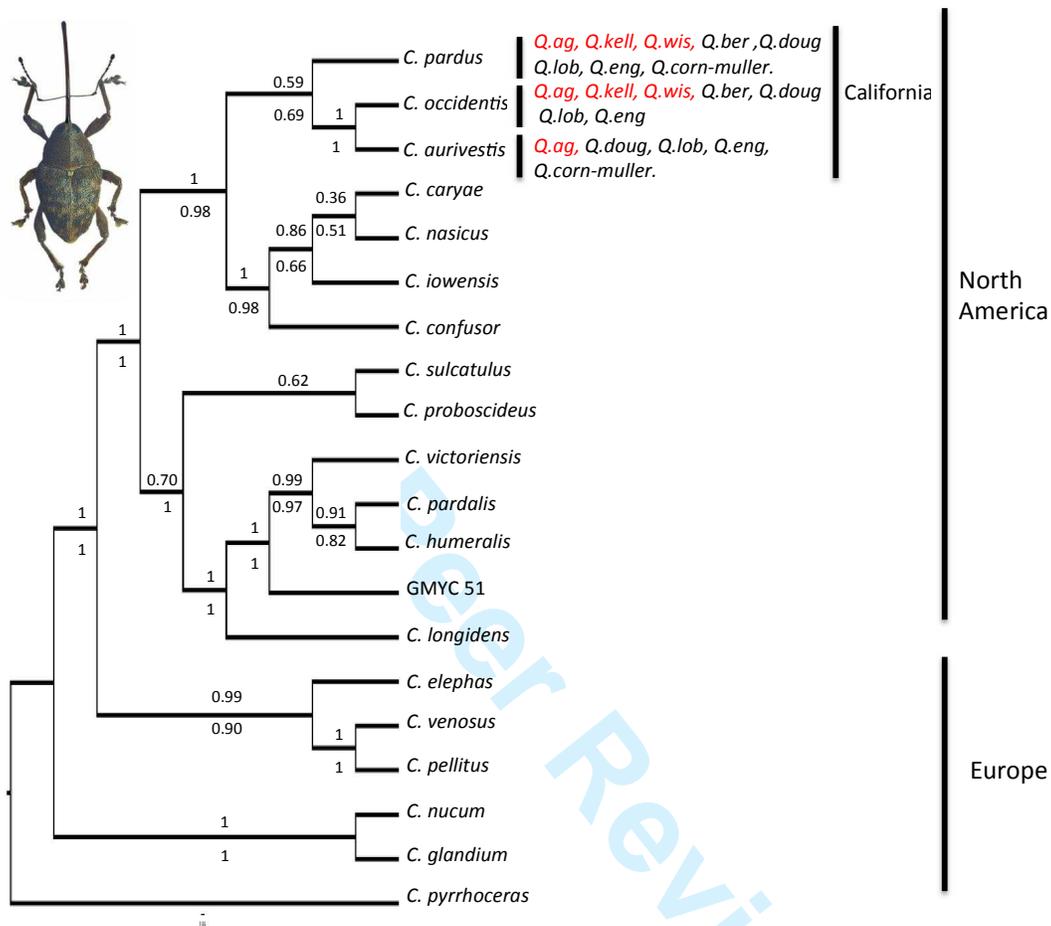
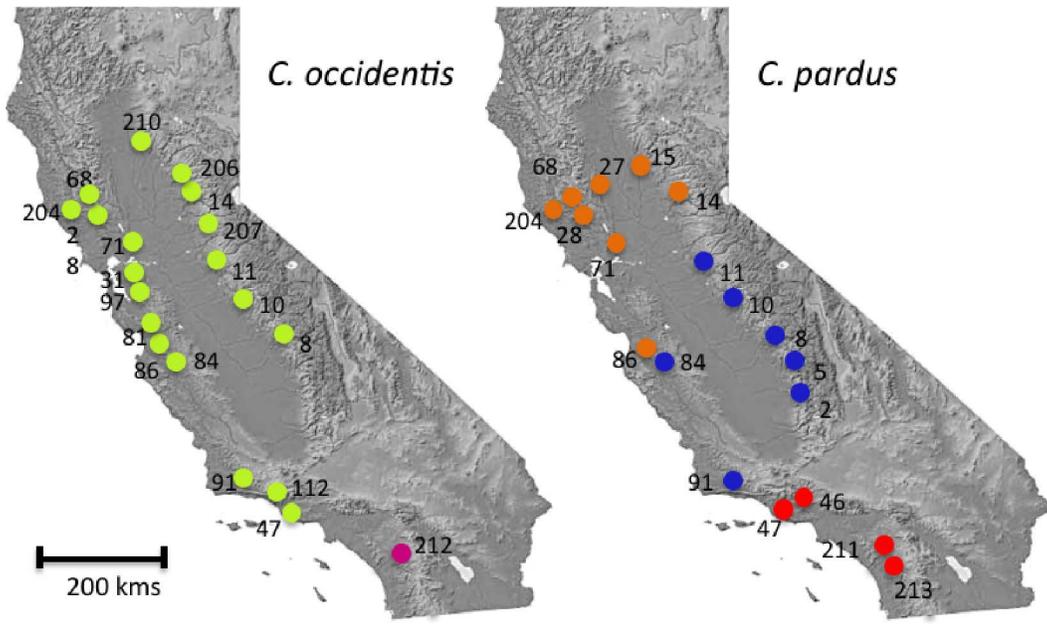


Figure 2



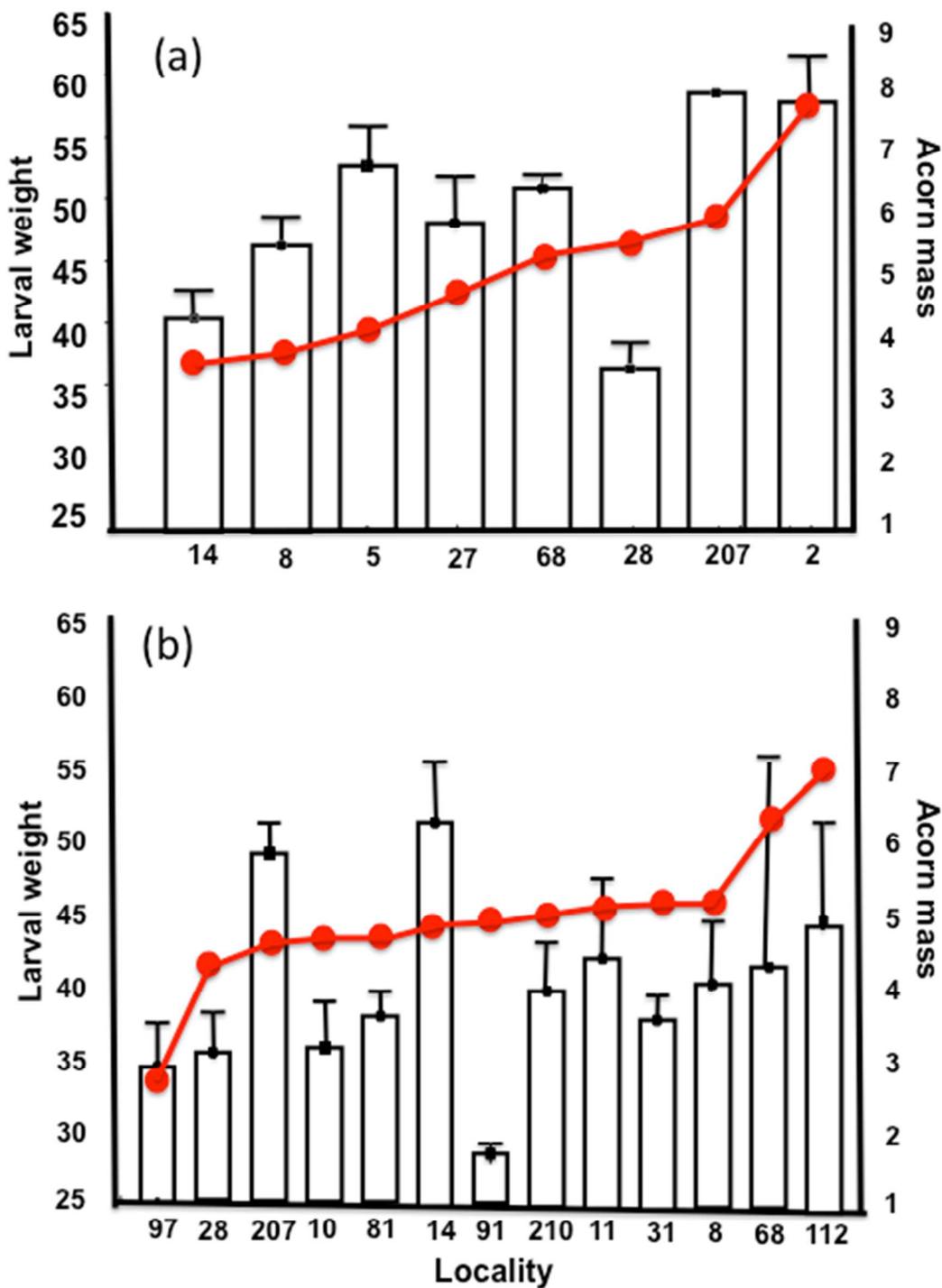
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Figure 3



Review

Figure 4



Journal of Biogeography

SUPPORTING INFORMATION

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

Raúl Bonal, Josep M. Espelta, Alberto Muñoz, Joaquín Ortego, José Miguel Aparicio, Keith Gaddis and Victoria L. Sork

Appendix S1 Locality code, geographical location, host oak species and number of collected individuals for each species of *Curculio* in California, USA.

Locality	Latitude	Longitude	<i>Quercus</i> species	<i>C. aurivestis</i>	<i>C. occidentis</i>	<i>C. pardus</i>
2	36.060	-119.034	<i>Q. lobata</i>	0	0	16
5	36.476	-119.121	<i>Q. lobata</i>	0	0	20
8	36.725	-119.459	<i>Q. lobata</i>	0	5	12
10	37.462	-119.880	<i>Q. lobata</i> , <i>Q. douglasii</i> , <i>Q. wislizenii</i>	1	9	8
11	37.979	-120.388	<i>Q. lobata</i> , <i>Q. kelloggii</i>	0	14	4
14	38.996	-121.108	<i>Q. lobata</i> , <i>Q. kelloggii</i> , <i>Q. wislizenii</i>	0	11	20
15	39.227	-121.422	<i>Q. douglasii</i>	0	0	16
17	39.711	-122.004	<i>Q. lobata</i>	0	2	0
27	39.089	-122.346	<i>Q. lobata</i> , <i>Q. douglasii</i>	0	0	19
28	38.748	-122.618	<i>Q. lobata</i>	0	10	5
31	37.865	-122.034	<i>Q. lobata</i>	0	18	0
46	34.412	-118.570	<i>Q. lobata</i> , <i>Q. agrifolia</i>	0	0	18
47	34.187	-118.890	<i>Q. lobata</i> , <i>Q. agrifolia</i>	0	15	4
68	39.043	-122.775	<i>Q. lobata</i> , <i>Q. douglasii</i>	0	6	14
71	38.493	-122.148	<i>Q. douglasii</i> , <i>Q. wislizenii</i> , <i>Q. berberidifolia</i>	0	23	11
81	36.834	-121.552	<i>Q. lobata</i> , <i>Q. kelloggii</i> , <i>Q. agrifolia</i>	2	31	0
84	36.099	-121.151	<i>Q. agrifolia</i>	0	7	4
86	36.385	-121.558	<i>Q. douglasii</i> , <i>Q. agrifolia</i>	4	14	11
91	34.699	-120.040	<i>Q. lobata</i> , <i>Q. agrifolia</i> , <i>Q. douglasii</i>	0	28	6
97	37.354	-121.741	<i>Q. lobata</i> , <i>Q. douglasii</i> , <i>Q. agrifolia</i>	8	19	1
112	34.455	-119.230	<i>Q. lobata</i> , <i>Q. agrifolia</i>	0	4	2
204	38.985	-122.970	<i>Q. douglasii</i> , <i>Q. berberidifolia</i>	2	29	4
206	39.210	-121.300	<i>Q. lobata</i>	0	7	0
207	38.486	-120.846	<i>Q. lobata</i>	0	18	1
210	39.636	-121.946	<i>Q. lobata</i>	3	9	1
211	33.272	-117.183	<i>Q. berberidifolia</i> , <i>Q. engelmannii</i>	0	0	10
212	33.275	-116.623	<i>Q. agrifolia</i> , <i>Q. engelmannii</i> , <i>Q. cornell.-mulleri</i>	4	5	0
213	33.235	-117.022	<i>Q. berberidifolia</i> , <i>Q. engelmannii</i>	0	0	10
214	33.065	-116.401	<i>Q. engelmannii</i> , <i>Q. cornellius mulleri</i>	1	0	2
215	33.042	-116.325	<i>Q. engelmannii</i>	0	1	0