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A novel approach to an old problem: tracking dispersed seeds

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Abstract

Animals are the principal vectors of dispersal for a large number of plant species. Unfortunately it is not easy to discern their movement patterns or the fate of their dispersed seeds. Many animals transport seeds by consuming them and then, some time later, defecating them. Others gather seeds and then store them for later consumption. Both circumstances lead to a set of seeds that have been dispersed in a clumped pattern, which offers a unique opportunity to assess seed movements. We introduce a novel approach that uses maternally inherited seed tissue to quantify the genetic structure of dispersed seed pools. This direct approach measures the genetic variability within and among seed pools, and estimates the scale of seed movement, without requiring a highly polymorphic battery of markers or the location and genotypes of all possible seed parents. We demonstrate this approach with the specific case of seed transport of valley oak (*Quercus lobata*) acorns by acorn woodpeckers (*Melanerpes formicivorus*). These territorial birds store acorns in drilled holes in the bark of trees, called granaries. We sampled stored acorns from different granaries, extracted DNA from the maternally inherited pericarp, and then assessed individuals for three microsatellite markers. We found extremely high genetic structure among granaries, a low number of effective seed donors per granary, and restricted seed movement. A maternity analysis performed on the same sample with seven microsatellites confirms acorn transport is limited to approximately 100-m radius. Our findings provide insight into the foraging and seed-dispersal behaviour of acorn woodpeckers, with an approach that can be widely extended to other systems.

Keywords: acorn woodpecker, maternal tissue, maternity analysis, seed movement, seed pool, valley oak acorn

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Introduction

Seed movement is a critical process for gene flow among populations and colonization of new sites. Yet, tracking seed movement can be a difficult task. Seeds can be tracked either by following their physical movement from point of source to point of fall (e.g. Levey & Sargent 2000; Gomez 2003), or by finding a dispersed seed and then retracing its path back to its maternal source (e.g. Dow & Ashley 1996; Godoy & Jordano 2001; Grace *et al*. 2004). Both approaches are somewhat feasible for abiotic seed dispersal via wind (Bullock & Clarke 2000; Nathan *et al*. 2002; Soons *et al*. 2004; Wagner *et al*. 2004) or water (Campbell *et al*. 2002), but extremely challenging for dispersal by animals (Dalling *et al*. 2002; Wehncke *et al*. 2003) because it is difficult to follow dispersers and to locate the transported seeds (e.g. Levey & Sargent 2000; Holbrook *et al*. 2002). Most likely, the majority of seeds will be dispersed locally (Levin & Kerster 1974), usually resulting in a leptokurtic distribution (e.g. Wenny & Levey 1998; Westcott & Graham 2000; Godoy & Jordano 2001; Gomez 2003; Jansen *et al*. 2004), although for animal dispersal the mean and shape of the curve will vary among dispersal agents and plant species. The remaining portion will be dispersed over long distances, reflecting either a long 'tail' of the distribution or an

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episodic event. Because of the genetic and demographic importance of both local and distant dispersal in animal-dispersed plants, it is critical to find ways to describe this process.

Molecular markers offer a useful tool for studying seed movement (Ouborg *et al*. 1999; Sork *et al*. 1999; Cain *et al*. 2000). One approach, called maternity analysis, uses the seedling to infer the parents through a maximum-likelihood approach (for a review on parentage analysis methods, see Jones & Ardren 2003). The caveat of this approach is that we have access to the parent pair, but we cannot distinguish the mother from the father. An exciting breakthrough for the study of dispersal in angiosperms is the use of genotypes derived from maternally inherited seed tissue in the seed coat (Godoy & Jordano 2001). These seed coat genotypes can be maternity analysed to identify the maternal parent of the dispersed seed. Such an approach is most effective for studies with high-resolution genetic markers, large sample sizes of progeny, genotypes of all potential seed parents, and spatial locations of those potential parents. When DNA quality is poor, often the case from the seed coat, or, when genotypes for multiple loci are not available, it is difficult to conduct maternity analysis. Additionally, when potential mothers are too widely distributed to locate, maternity analysis is also not practical, despite good genetic resolution. Nonetheless, molecular markers can provide valuable information about the genetic consequences of seed dispersal, even when a maternity analysis is not feasible.

Here we introduce a seed pool structure approach that allows us to study seed movement by examining the genetic consequences of dispersal. Our approach is an extension of a pollen pool approach that estimates the effective number of pollen donors, measured as a *Probability of Paternal Identity* (PPI) (Smouse & Robledo-Arnuncio 2005). Using exactly analogous logic, our approach will measure the *Probability of Maternal Identity* (PMI), which gauges the degree of seed pool structure and estimates the effective number of contributing mothers per seed pool. This method requires sampling sets of seeds, either from naturally occurring seed pools, such as seed storage sites or nesting sites, or from artificially placed sampling sites, such as seed traps. Within the seed pool or stratum level the PMI measures maternal diversity, while at the among stratum level it estimates the seed pool overlap between pairs of strata, yielding an estimate of maternal sharing. An analysis of the spatial extent of seed pool overlap will reveal the scale of seed dispersal. The novelty of this approach is that it only requires the maternal designation of seeds from patches, but not the location of those maternal sources.

To illustrate this new approach, we present a study of acorn woodpeckers (*Melanerpes formicivorus*), territorial birds that store acorns in drilled holes in the trunks of seed storage trees, called 'granaries'. The granaries constitute identifiable seed pools, and offer a unique opportunity to ask whether woodpeckers are moving acorns over long or short distances. On one hand, these birds can fly over 10 km per day (Koenig *et al*. 1996b) and if acorns are frequently carried over such distances, then the seed pools of the granaries should be nondifferentiated. On the other hand, if the birds collect acorns primarily within their own territories (the size of which ranges from 2 to 6 ha in California, MacRoberts & MacRoberts 1976; Swearingen 1977), then dispersal should be quite localized, and the granary seed pools should be highly differentiated, with a small effective number of seed donors per granary.

The goals of this study were to introduce a new approach to the analysis of seed movement and to apply this method to the study of a bird species capable of long-distance dispersal. Using maternally inherited tissue (the pericarp) from granary-collected acorns, we address four questions: (i) Are the granary seed pools genetically differentiated? (ii) What is the effective number of seed sources per granary (N_{em})? (iii) What is the spatial scale of seed movement? (iv) Based on the genetic findings, what can we infer about foraging and seed-dispersal behaviour of birds?

Materials and methods

Study site

Our study site is located at Sedgwick Reserve in the Santa Ynez Valley of Santa Barbara County (California, USA, 34°42′N, 120°02′W) and encompasses about 180 ha (900 × 2000 m). Three oak species coexist in the oak–savannah landscape of the Reserve: valley oak (*Quercus lobata*), blue oak (*Quercus douglasii*), and coast live oak (*Quercus agrifolia*). For more details about the study site, see Sork *et al*. (2002a, b). For details about acorn production since 1994, see Koenig *et al*. (1994, 1996a).

Study species

The species chosen for this study is California valley oak (*Quercus lobata* Née), which has suffered serious range reduction over the past 200 years (Swiecki & Bernhardt 1991), and which is also typified by a lack of recruitment within local populations (Muick & Bartolome 1988; Brown & Davis 1991). *Q. lobata* stem density is low, between 2 and 10 trees/ha. Like all oak species, valley oak produces monoecious flowers that are wind pollinated in the spring, with the acorns maturing in the fall. Acorns are dispersed by gravity, acorn woodpeckers, western scrub jays, and possibly small rodents.

Acorn woodpeckers are the focal dispersal agents in this study (Fig. 1). They are present throughout the year at our study site, living in family groups in permanent territories.

Fig. 1 Montage of an acorn woodpecker (*Melanerpes formicivorus*) and a granary (*Quercus lobata*) (photos by A. R. Pluess and D. Grivet).

The diet of acorn woodpeckers includes a wide range of insects and fruits, but they are also dependent on acorns, which are critically important to almost all aspects of their behaviour and ecology (for a review, see Koenig & Benedict 2002). They harvest acorns directly from oaks in the fall, and store them, often by the thousands in granaries, most frequently in the tree trunks of *Q. lobata* at our study site. Acorns are usually stored individually in holes drilled by the birds expressly for this purpose. Holes are used year after year and additional ones accumulate over time.

Sampling

In January 2003, we collected 215 acorns of *Q. lobata* (6–19 per granary) from 17 granaries, covering an area of 123.6 ha throughout the Figueroa valley of Sedgwick Reserve (Fig. 2A). In general, each of the granaries (or, in some cases, a pair of adjacent granaries) constitutes a storage repository for each acorn woodpecker territory, inhabited and defended by a single-family group (MacRoberts 1970). Acorns randomly collected within each granary were cleaned and stored at room temperature, pending DNA isolation from the pericarp, a somatic maternal tissue in *Quercus* (Ziegenhagen *et al*. 2003). For the maternity analysis, we expanded our previous adult sample size from 191 (Dutech *et al*. 2005) to 327 adult trees located in Figueroa Creek valley. This sampling includes at least all the adults located within a 150-m radius of each granary. This sampling zone was chosen, because it is close to the acorn woodpeckers' average foraging range (W. Koenig, personal communication).

DNA isolation and amplification

When we collected the acorns, the pericarps were mature (brown) and dry (woody). Due to the presence of tannins (polyphenolic compounds that can interfere with both the isolation and subsequent utilization of DNA), the DNA extracted was generally difficult to amplify, and/or fragmented, resulting in low copy numbers of the target DNA. About 50 mg of pericarp tissue were cut into small pieces in a microtube and soaked in the extraction buffer for at least 30 min before proceeding to the DNA extraction. We used the Mixer Mill MM301 (Retsch) with a tungsten ball to grind the pericarps in powder, and the DNeasy Plant Mini Kit (QIAGEN) to extract the DNA. Due to the poor quality of the extracted DNA, we only used three primers for the seed pool approach, each of them selected for its reliability and designed to amplify a particular hypervariable region in *Quercus* (SSR: single strand repeats or microsatellites) of the nuclear genome: MSQ4 (Dow *et al*. 1995), QpZAG1/5 (Steinkellner *et al*. 1997), and QrZAG20 (Kampfer *et al*. 1998). We also used partial information on four additional (less reliable) primers to extend the genetic coverage of our maternity analysis: QpZag9 and QpZag36 (Steinkellner *et al*. 1997), QrZag11 (Kampfer *et al*. 1998) and QM50 (Isagi & Suhandono 1997). We have optimized the polymerase chain reaction (PCR) conditions for pericarp DNA, which involved the following (touchdown) profile: 12 s at 95 °C, followed by 17 cycles of 30 s denaturing at 94 °C, 1 min annealing from 66 °C to 50 °C, and 45 s extension at 72 °C, followed by 19 cycles of 30 s denaturing at 89 °C, 1 min annealing at 50 °C, and 45 s extension at 72 °C, with a final extension step of 72 °C for 3 min. The reaction solution contained four dNTPs (each 0.4 mM), variable concentrations of $MgCl_2$ (6.5 mM for QpZAG1/5 and QpZAG20, 3.5 mM for MSQ4, 2.5 mM for QpZag9, QpZag36, QrZag11, QM50), 0.6 μM of each primer, 1× reaction buffer (Applied Biosystems), 0.75 mg/mL of bovine serum albumin, 25 ng DNA and 0.5 unit of *Taq* polymerase (Ampli*Taq* Gold, Applied Biosystems). For the maternity analysis, the leaves of the additional adult trees were extracted with the QIAGEN Kit and PCR conditions were performed as described in Pluess *et al*. (in prep.).

We amplified acorn and adult leaf tissue, as well as a negative DNA isolation control (tissue replaced by water) and a negative PCR control (PCR mixture without DNA), to check for contamination. Each amplification was performed twice to check for the reliability of the genotypes. When two runs were not congruent (35% of our acorn

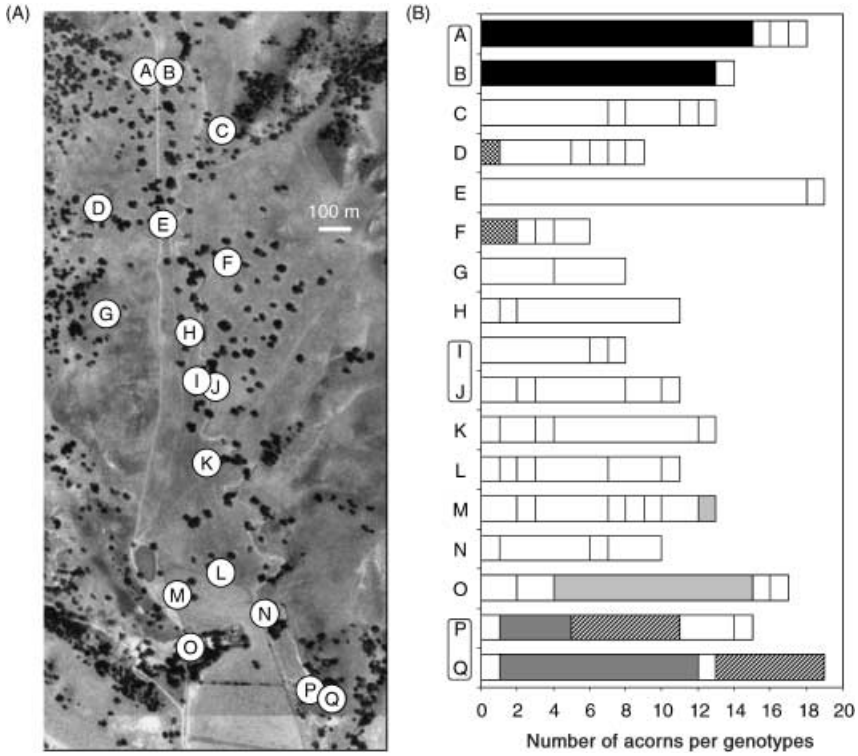

Fig. 2 Location and genetic diversity of the granary stratum based on three microsatellite loci. (A) Map of locations of 17 granaries (A–Q) covering an area of 180 ha (including all trees located within 150 m from each granary) at Sedgwick Reserve, sampled during January 2003 (aerial photo by F.W. Davis). Other trees correspond to a mix of the three oak species (*Quercus lobata*, *Quercus douglasii*, and *Quercus agrifolia*). (B) Histogram of the acorn (maternal) genotypes found within and among granaries. The filled histograms correspond to duplicate genotypes, whereas the empty histograms correspond to unique genotypes. The bracketed granaries correspond to pairs of granaries located within 30 m.

duplicates), it was because only one allele amplified in one trial and heterozygote amplified in the other. In those cases, we chose the heterozygote genotype to avoid carrying partial null alleles. We analysed an aliquot of each PCR product with an ABI 3700 automatic sequencer, and genotyped them based on the statistical packages of the UCLA Sequencing and Genotyping Core Facility (for details see, http://www.genetics.ucla.edu/sequening/index.php).

Probability of maternal identity

Smouse & Robledo-Arnuncio (2005, henceforth SR-A) have deployed a method of gauging the genetic structure of the pollen pool (within and among mothers) as the *Probability of Paternal Identity* (PPI). Having used paternity analysis to determine the genotypes of the fathers, they used a direct counting approach to estimate the probability that a random pair of progeny, drawn from the gth mother, have the same father. We adapt the same statistical machinery here to estimate the *Probability of Maternal Identity* (PMI), formally, the probability of identical seed donors (henceforth, mothers), drawn from two random seeds from the same granary. Consider a total set of K mothers recovered from the entire set of N seeds, drawn from G separate granaries. Let the sample size from the gth granary be n_g, and let the number of seeds provided by the kth mother for the gth granary be x_{gk}, with $\Sigma_k x_{gk} = n_g$. Following SR-A, there are two slightly different estimators of PMI for the gth granary (q_{gg}, r_{gg}), defined as follows:

$$q_{gg} = \sum_{k=1}^{K} \left(\frac{x_{gk}}{n_g} \right)^2 , \qquad \text{(eqn 1a)}$$

$$r_{gg} = \sum_{k=1}^{K} \frac{x_{gk}(x_{gk} - 1)}{n_g (n_g - 1)} . \qquad \text{(eqn 1b)}$$

SR-A show that q_{gg}, the more traditional measure, has the smaller variance, but is biased. That bias is substantial for small n_g and small parametric values of PMI. On the other hand, r_{gg} has no bias and is an accurate estimate of PMI, but it has larger variance than q_{gg}. We are trading precision (variance) off against accuracy (bias) when choosing between them. SR-A recommend the mean squared error, MSE = variance + (bias)^2 as a criterion of choice. Careful evaluation reveals that MSE(q_{gg}) < MSE(r_{gg}) when PMI > 0.40 and MSE(q_{gg}) > MSE(r_{gg}) when PMI < 0.40, so we should use r_{gg} with PMI < 0.40 and q_{gg} with PMI > 0.40. For large sample sizes, there is very little difference in MSE between the two estimates, but sample sizes for individual granaries will typically be (and are certainly here) small, so the difference matters. We do not know PMI in advance, and will find it useful to carry both measures as we proceed.

For the small sample sizes we have for individual granaries in this study, the MSEs are large, and the corresponding PMI are neither accurate nor precise. Estimating the average PMI over granaries ($\bar{q}_0$, $\bar{r}_0$) makes more sense as this estimate presents a smaller MSE by virtue of greater

replication for the total set of granaries. We can obtain a pair of sample-size-weighted per-granary average estimates as follows:

$$\bar{q}_0 = \frac{\sum_{g=1}^{G} n_g^2 q_{gg}}{\sum_{g=1}^{G} n_g^2}, \qquad \text{(eqn 2a)}$$

$$\bar{r}_0 = \frac{\sum_{g=1}^{G} n_g (n_g - 1) r_{gg}}{\sum_{g=1}^{G} n_g (n_g - 1)}. \qquad \text{(eqn 2b)}$$

The estimate $\bar{q}_0$ is still biased, but both have smaller MSEs than the single-granary estimates.

The granaries are separated by 20–2000 m, and we expect more maternal overlap between neighbouring granaries than between those at opposite ends of the valley. To examine the pattern of intergranary maternal overlap, we estimate the PMI for two random seeds, one each from the gth and hth granaries, as a way of determining the extent to which acorn woodpeckers harvest seeds from the same maternal source, but for different granaries. It develops that the two measures are identical, so we have:

$$q_{gh} = \sum_{k=1}^{K} \left(\frac{x_{gk}}{n_g} \right) \cdot \left(\frac{x_{hk}}{n_h} \right) = r_{gh}, \qquad \text{(eqn 3a)}$$

for any particular pair of granaries, and

$$\bar{q}_{gh} = \frac{\sum_{g \neq h}^{G} n_g \cdot n_h \cdot q_{gh}}{\sum_{g \neq h}^{G} n_g \cdot n_h} = \bar{r}_{gh} \qquad \text{(eqn 3b)}$$

for the average pair of granaries. The seed pool structure of the collection of granaries is defined in terms of the q- and r-coefficients, both those within single granaries and those between pairs of granaries.

Effective number of seed donors

Following SR-A, we can derive the effective number of seed-(maternal)-donors from our estimate of PMI, either $N_{em} = (1/q_{gg})$ or $N_{em} = (1/r_{gg})$ for the gth granary, and either $N_{em} = (1/\bar{q}_0)$ or $N_{em} = (1/\bar{r}_0)$ for the average mother, depending on whether we ultimately use the q-measures or the r-measures.

Maternity analysis

To assess the utility of our three most reliable markers (MSQ4, QpZAG1/5 and QrZAG20) for maternal identification, we computed the multilocus probability of genetic identity (P_{ID}) for the 327 mother trees sampled and genotyped. Paetkau *et al*. (1998) provide a theoretical expectation, based on multilocus Hardy–Weinberg mathematics, randomly spread across the landscape, and using their equation,

$$P_{ID} = \frac{n^3(2a_2^2 - a_4) - 2n^2(a_3 + 2a_2) + n(9a_2 + 2) - 6}{(n-1)(n-2)(n-3)}, \qquad \text{(eqn 4)}$$

where n is the sample size, with $a_i = \Sigma\, p_j^i$ and P_j is the frequency of the ith allele, we found the overall $P_{ID} = 0.00096$. We have established elsewhere (Dutech *et al*. 2005) that there is subtle genetic structure among the adults at the Figueroa Creek site, with nearby pairs of individuals being slightly more similar genetically and more distant pairs being slightly less similar than average. The adult collection of genotypes is not (quite) randomly distributed across the landscape, and we judged it wise to accommodate that fact. In our collection of 327 mother trees, 271 display no missing data for three-locus genotypes; among them there are 243 unique genotypes (one realization each), 22 matched pairs, 5 matched trios and 1 matched quartet. This total collection lead to a $P_{ID} = 0.001175$, slightly above the theoretical value ($P_{ID} = 0.00095$ for 271 mother trees) due to the violated randomness assumption, but clearly P_{ID} is very small.

The addition of four less reliable loci would decrease this P_{ID} value even further, basically reducing each of the matched sets to a collection of unique seven-locus genotypes, but we only have partial information on these other loci. Thus, for our main study of seed pool structure, we have used our three best loci only. Even with the limited genetic resolution at our disposal, the seed pool structure results are compelling. In the Discussion we will return to the seven-locus genotypes that we do have, to determine whether we can improve our genetic inference just a bit on the locations of the adults contributing to the granaries. For both analyses, we compared seed coat with adult genotypes, using software developed by Brian Dolan (Biomathematics, UCLA; available upon request), matching seed coat and maternal genotypes, on the basis of whatever genetic information we could bring to bear in a particular case.

Results

Using the software GENEPOP version 3.4 (Raymond & Rousset 1995), we summarized the diversity of the three microsatellite loci, finding that MSQ4, QpZag1/5, and QrZag20 have 19, 8 and 9 alleles, respectively. The values

of observed heterozygosity were 0.80, 0.51, and 0.41, respectively. We do not report the diversity of the four additional microsatellite loci, as they give partial genetic information (information on all these markers are available in Dutech *et al*. 2005; Pluess *et al*. in prep.). Using the three most reliable loci, with no missing data (MSQ4, QpZag1/5 and QrZag20), we detected 68 unique genotypes among the total sample of 215 acorns, with the numbers of different maternal genotypes per granary varying from two to eight (Fig. 2B). This number is a minimal estimate, because some of the replicated sets of matched three-locus maternal genotypes could almost surely be subdivided on the basis of additional loci, but these limitations of the genetic data do not detract from the interpretation of our results. We will show in the Discussion that additional genetic loci would help us refine our resolution even further, but that the essential answers are unequivocal with the limited information we already have.

Seed pool structure

The estimated PMI values (q_{gg} and r_{gg}) of random pairs of acorns, drawn from a single granary, vary among granaries (Table 1, Fig. 2B). Granaries vary both in the absolute numbers of different seed-donors (maternal richness) and in terms of the redundancy of their maternal contributions (maternal diversity). For example, granaries B ($q_{BB} = 0.867$, $r_{BB} = 0.857$) and E ($q_{EE} = 0.900$, $r_{EE} = 0.895$) display the highest PMI estimates, and they also exhibit low absolute numbers of maternal genotypes (two each), the most frequent of which is represented by 93% and 95% of the seeds, respectively. Granary A displays a lower PMI value ($q_{AA} = 0.704$, $r_{AA} = 0.686$), with four different maternal genotypes, but with one of those maternal genotypes represented by 83% of the seeds. By contrast, granary M exhibits eight different maternal genotypes, with the lowest PMI ($q_{MM} = 0.172$, $r_{MM} = 0.103$). Some of the single-granary estimates are greater than 0.40, and some are less, but on average, $\bar{q}_0 = 0.512$ and $\bar{r}_0 = 0.474$. As expected from the formal theory (SR-A), var $(\bar{q}_0) = 0.000726 <$ var $(\bar{r}_0) = 0.001580$, and in spite of some Bias $(\bar{q}_0) = 0.000242$, $\bar{q}_0$ is the better estimator [MSE $(\bar{q}_0) = 0.000968 <$ MSE $(\bar{r}_0) = 0.001580$]. In practice, however, the differences are small, and the patterns are the same with both measures (Table 1). The general pattern is for a small number of mothers contributing to any one granary, with one or two mothers providing most of the seeds. The data, analysed either with the *q*- or *r*-measure, suggests strongly that PMI ~ 1/2 for the average granary, and we thus have the remarkable result that the *effective number of mothers* contributing to any one granary is on the order of $N_{em} \sim 2$.

Table 1 Summary of probabilities of maternal identities (PMI) estimated with the biased *q*-estimator and the unbiased *r*-estimator (see text for details). PMI are given for single granary (q_{gg}, r_{gg}) and for the average within granary ($\bar{q}_0$, $\bar{r}_0$). The root mean squared errors (rMSE) are provided for the averaged estimators

q-estimator		*r*-estimator	
q_{AA}	0.704	r_{AA}	0.686
q_{BB}	0.867	r_{BB}	0.857
q_{CC}	0.361	r_{CC}	0.308
q_{DD}	0.259	r_{DD}	0.167
q_{EE}	0.900	r_{EE}	0.895
q_{FF}	0.278	r_{FF}	0.133
q_{GG}	0.500	r_{GG}	0.429
q_{HH}	0.686	r_{HH}	0.655
q_{II}	0.594	r_{II}	0.536
q_{JJ}	0.289	r_{JJ}	0.218
q_{KK}	0.420	r_{KK}	0.372
q_{LL}	0.240	r_{LL}	0.164
q_{MM}	0.172	r_{MM}	0.103
q_{NN}	0.360	r_{NN}	0.289
q_{OO}	0.453	r_{OO}	0.419
q_{PP}	0.280	r_{PP}	0.229
q_{QQ}	0.440	r_{QQ}	0.409
Average within granary			
q_0	0.512	r_0	0.474
rMSE(q_0)	0.031	**rMSE(r_0)**	0.037

Maternal overlap

In general, each granary contained acorns from different trees. We found only four specific examples of shared maternal genotypes among $G(G-1)/2 = 136$ pairs of granaries (Fig. 2B): granary pair A-B ($N = 32$), located 33 m apart, share one maternal genotype; granary pair P-Q ($N = 34$), located 23 m apart, share two maternal genotypes; granary pair M-O ($N = 30$), located 147 m apart, share one maternal genotype; and granary pair D-F ($N = 15$), located 356 m apart, share one maternal genotype. For these four examples, the rate of maternal overlap (i.e. the probability that two acorns, each drawn at random from a different granary, have the same mother) varies between granary pairs (Table 2); it is high for pair A-B ($q_{AB} = 0.774 = r_{AB}$), moderate for pair P-Q

Table 2 Probabilities of maternal identity across granaries (q_{ij}, r_{ij}) and for the average granaries (ave. q_{ij}, ave. r_{ij}). For the computation of the average across the granaries, the pairs that did not share any genotypes were equaled to zero

q–*r* estimators	
$q_{AB} = r_{AB}$	0.774
$q_{MO} = r_{MO}$	0.050
$q_{DF} = r_{DF}$	0.037
$q_{PQ} = r_{PQ}$	0.281
ave. q_{ij} = ave. r_{ij}	0.007

($q_{PQ} = 0.281 = r_{PQ}$), and small for pairs D-F and M-O ($q_{DF} = 0.037 = r_{DF}$ and $q_{MO} = 0.050 = r_{MO}$). Averaged over all 136 pairs of granaries, including the 132 observable nonoverlaps, we estimate the average rate of cross-granary maternal matching to be $\bar{q}_{gh} = 0.0067 = \bar{r}_{gh}$. Even with the few exceptions noted, the data tell us that the seed pool for these granaries is highly structured (Table 1). An examination of the four exceptions noted above tells its own story, to which we will return below.

Discussion

We found that the granary seed pools were highly differentiated during the year studied, which, if one assumes that seed dispersal is leptokurtic (Levin & Kerster 1974), suggests that seed transport by acorn woodpeckers is very restricted. Using a seed pool structure approach, we detected a substantial structure among granaries that were all located within a 2-km stretch of valley. The fact that we rarely observed the same maternal genotype in more than one granary is evidence of local seed movement. In fact, in the four cases detected among the 136 granary pairs, the maximum number of shared maternal genotypes was two. An alternative explanation that woodpeckers moved acorns far, but from few trees, is consistent with the observed seed pool structure but incongruent with acorn woodpecker behaviour (cf. discussion below) and not supported by additional analyses, which we present below.

One goal of this study was to assess the effective number of seed sources per granary as this information tells us the genetic consequences of seed dispersal. We found a limited number of unique maternal genotypes per granary: 4–5 genotypes on average, with the effective number of mothers $N_{em} \sim 2$ individuals. This limited number of effective seed sources may be caused by the foraging behaviour of acorn woodpeckers. Thus, to the extent that acorn woodpeckers disperse seeds incidentally as they move them from seed source to granary, the impact of these birds is not likely to increase the neighbourhood size of the tree populations.

In retrospect, such restricted acorn movement seems very consistent with optimal foraging theory, which predicts that animals should forage to maximize energy gain at the lowest energy cost (Schoener 1971). Hence, a woodpecker should collect acorns from the closest trees to its granary. It is also relevant that acorn woodpeckers live in permanent territories that include one focal granary (or a set of adjacent granaries) and the surrounding area (Koenig *et al*. 1995). Family groups defend their granaries against intruders. Smith & Reichman (1984) suggested that acorn woodpeckers protect their focal granaries as well as the nearby trees from which they collect acorns with the assistance of close genetic relatives, who also benefit from the availability of stored acorns. Thus, the consequence of the foraging and territorial behaviour is that the granaries within a single territory contain a low number of different maternal genotypes.

The virtual nonoverlap in acorn genotypes among granaries in different territories is consistent with woodpecker behavioural observations that report no territorial overlap between neighbouring family groups (MacRoberts & MacRoberts 1976; Mumme & De Queiroz 1985). Instead, territorial boundaries are well defended against intruders (MacRoberts & MacRoberts 1976), which decreases the rate of neighbouring kin groups foraging in each other's territory. Restricted acorn foraging is the generality, despite occasional observations of birds flying at least 300 m to find and collect acorns for storage, most often occurring in years when the acorn crop is patchy and acorns are not available on some territories (MacRoberts & MacRoberts 1976). Moreover, in spite of occasional observations that birds fly up to 1 km from their own territories during fall acorn-storing season (W.D. Koenig, personal observation) and can fly up to 10 km or more within a day (Koenig *et al*. 1996b), it is not apparent that these forays result in meaningful long-distance seed movement.

The third objective of our study was to assess the spatial scale of seed movement to see if acorn woodpeckers move acorns far away or only within their own territory. In theory, modelling the shared maternal genotypes (r_{gh}) as a function of intergranary distances can give us a detailed picture of the spatial genetic structure of the acorns. If a sufficient number of granary pairs share some maternal genotypes, then it is possible to use the relationship between the proportion of maternal genotypes shared between granary pairs and the distance between these pairs to analyse how maternal genetic relatedness evolves with the intergranary distance. In practice for our study, only four pairs of granaries share maternal trees and do not allow us to assess the scale acorn movement. Nonetheless, the analysis of these four overlapping granary pairs gives us additional information on the bird behaviour. The geographically closest pairs (located at 23 and 33 m) show the most overlap ($r_{PQ} = 0.281$ and $r_{AB} = 0.774$, respectively), whereas the two more distant pairs (located 147 m and 356 m apart, respectively) show substantially less overlap ($r_{MO} = 0.050$ and $r_{DF} = 0.037$). Thus, we can say the scale of seed dispersal is very local, but these cases do not provide a useful demonstration on how the pairwise genetic distances allow us to infer the scale of seed movement.

The approach we introduce here should be useful not only because of what it tells us about seed movement, but also because of what it reveals about bird movement. In cases where the animals are hard to track or foraging is difficult to observe, one can use seed pool structure information to make inference about the animal. In our case, we learned that acorn woodpeckers collect acorns from few trees located within their territories, indicating that acorn woodpeckers did not forage broadly during this season.

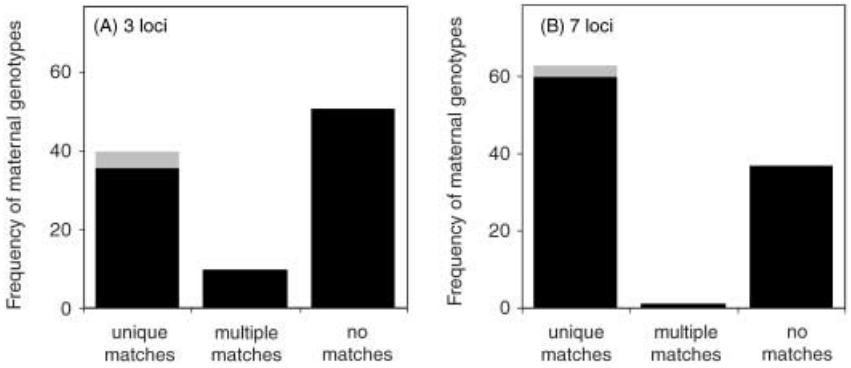

Fig. 3 Identification of the maternal seed donors. The histograms represent the percentage of the maternal genotypes cumulated across the 17 granaries for 3 loci (A) vs. 7 loci (B) breaking down into three categories: (i) acorn matching with unique maternal tree located within 150 m from the granary (black symbol) and beyond 150 m from the granary (grey symbol), (ii) acorn matching with multiple maternal trees, and (iii) acorns that do not match with any of the 327 maternal trees.

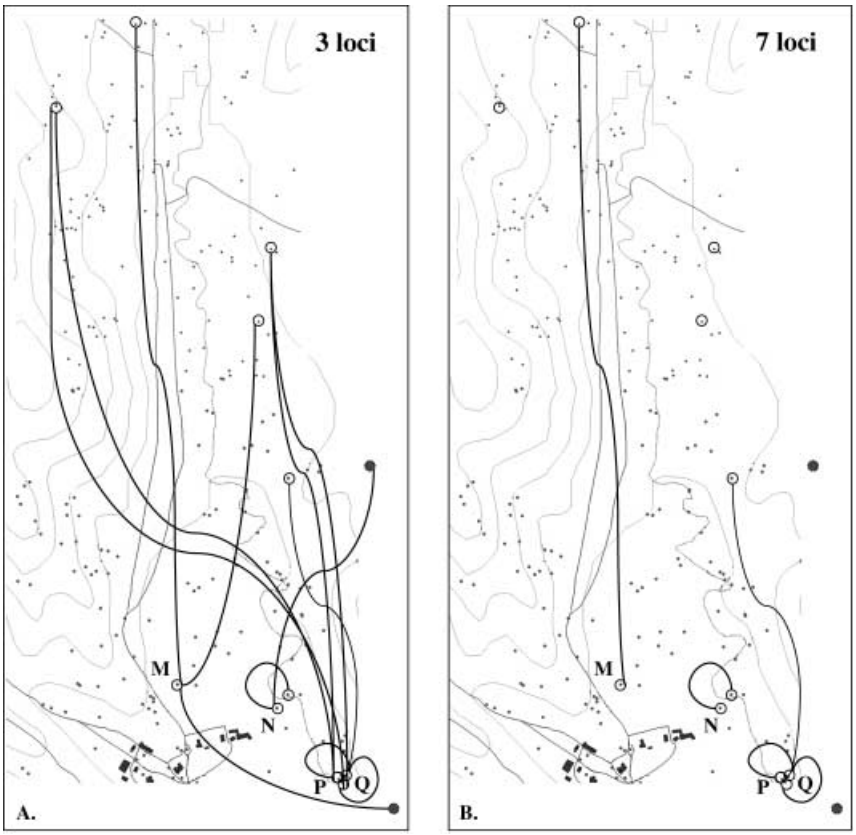

Fig. 4 Location of the maternal seed donors. Maps illustrate the cases of multiple matches using 3 loci (A) resolved when using 7 loci (B). The granaries are labelled as in Fig. 2. Circles with plain line correspond to adult trees located on our map; grey circles correspond to adult trees located outside our map (their position indicates the approximate direction of their location in the field). Connectors represent the seed movement from seed donors towards granaries (M, N, P, Q).

This approach could reveal the details of where and how frequently other animals forage in a given area.

What if the maternal seed sources can be localized?

So far, this study has focused on the seed pool structure approach to the study of seed movement while no information on the localization of the maternal sources was integrated. To have a more accurate picture of the scale of seed movement and to 'ground truth' our results, we conducted a direct maternity approach, such as Godoy & Jordano (2001). When only using our three most robust loci, we could localize unique maternal trees for only 39% of the acorns. The 61% remaining could not be identified because their maternal tree had not been genotyped for all seven loci or their maternal tree was outside the sampled area (51%) or because they matched with multiple trees (10%) (Fig. 3A). Expanding our genetic battery to seven loci allowed us to localize the maternal trees for a larger number of acorns (64%) and to reduce the nonmatching cases to 35% (Fig. 3B). It also allowed us to have a better sense of seed movement: with three loci, all the multiple matches from granaries M, N, P and Q show several long-distance seed movement events (Fig. 4A). When we improved the genetic resolution, the long-distance events occurred only twice (Fig. 4B). In both cases, acorn

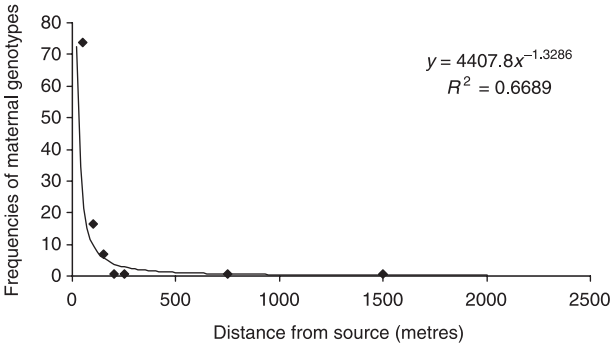

Fig. 5 Frequency distribution of maternal tree dispersal distances cumulated across the 17 granaries. The results correspond to the unique maternal trees identified with 7 loci. The line represents the power function that best describes these points based on the equation of function given in figure; corresponding R^2 indicated on the figure.

woodpeckers flew to trees located across relatively low-density area, which may have minimized interactions with the birds from other territories.

Based on the additional loci, we can have access to the scale of woodpecker-mediated seed movement: from the source-obvious acorns, 97% have a maternal source within 150 m of the granary (Figs 3B and 5). This scale is compatible with the observed territory sizes of foraging acorn woodpeckers. Koenig (unpublished data) reports a mean distance between source and granary for Valley oak acorns of 48 ± 15 m and 105 ± 11 m, respectively, for two different acorn woodpecker family groups at the Hasting Reservation, 300 km north of our study area, during fall 2004. Amid these within-territory acorn movements, we found one case where an acorn from granary M was collected from an adjacent maternal source O that is also a granary. This example may illustrate a case where acorn woodpeckers collect acorns from another adjacent territory.

In addition to the scale of acorn movement, the shape of the dispersal curve informs us on the frequencies of short- and long-distance events. The dispersal pattern is leptokurtic (the distribution is peaked and has fat tail) and fits a fat-tailed power function, which means that the curve drops less quickly than one would expect for an exponential distribution (Fig. 5). If these acorns were to result in seedling establishment, we would see strong genetic autocorrelation. Thus, from a genetic perspective, seed movement is restricted and neighbourhood size due to this process would be small. We also point out that long-distance dispersal within the landscape sometimes occurs, and it could be important from a colonization viewpoint. We are cautious about over-interpreting our findings, because (i) we know little about the seed transport to seedling establishment transition for woodpecker-mediated dispersal and (ii) our sample sizes are limited. Nonetheless, this analysis illustrates what we learn when we can localize the mothers and it also illustrates that the findings from this more refined analysis are consistent with the more widely feasible seed pool structure approach.

Conclusion

Our findings constitute a first step toward using seed pool maternal structure for the study of seed movements and storage patterns for the acorn woodpecker. Increasing our sample size (more acorns per granary) will allow us to investigate further this woodpecker-mediated seed movement. Our next step will be to study the impact of acorn crop size (both good and bad years) on woodpecker foraging behaviour. In bad years, birds may collect acorns from farther away, in an effort to gather enough acorns to stock their granaries, a pattern that would result in higher maternal variation within granaries and in spatially more extensive seed movement. Another area to explore will be to see how accurately the seed movement pattern reflects the disperser movement pattern. A set of coordinated foraging observations on the woodpeckers, coupled with larger samples of acorns from particular granaries, would improve our understanding of the relationship between seed dispersal and the behavioural ecology of dispersers.

The novelty of the seed pool approach is that it allows the analysis of contemporary seed flow without the level of genetic resolution required for categorical maternity analysis, and without the necessity of locating and genotyping all possible seed parents. This robust approach is particularly well suited for conducting comparative research at different temporal and spatial scales and in different ecological settings. We present this new approach for consideration by evolutionary and conservation biologists who work with species for which it is challenging to characterize the potential mothers at large distances (because of low density) or to find them all (because of high species diversity). Finally, this seed pool approach can be applied to many animal-dispersers of seeds deposited in patches (e.g. Chavezramirez & Slack 1994; Notman *et al*. 1996; Fragoso 1997; Frost & Rydin 2000; Wehncke *et al*. 2003; Borchert 2004; Russo & Augspurger 2004), as well as other vectors leading to a patchy distribution of seeds. The novelty and usefulness is that we can use information on plant genetics to inform our studies of animal behaviour.

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This effort represents collaborative research, aimed at developing spatial approaches to contemporary pollen and seed movement. D.G.'s expertise is evolutionary dynamics of plant populations at contemporary and historical levels. V.L.S. studies plant evolutionary biology, population genetics and conservation; P.E.S. has interests in population genetics and biostatistical modelling.
