

Reproductive processes in two oak (*Quercus*) contact zones with different levels of hybridization

JOSEPH H. WILLIAMS*†, WILLIAM J. BOECKLEN†‡ & DANIEL J. HOWARD†

†Department of Biology, MSC 3AF, ‡Department of Experimental Statistics, MSC 3CQ, PO Box 30001, New Mexico State University, Las Cruces, NM 88003, USA

Patterns of reproductive isolation between two sympatric species of oaks, *Quercus gambelii* and *Q. grisea*, that exhibit strong ecological differentiation were examined. A full diallel cross using four trees of each species (i.e. all possible pollinations among eight trees) was performed. This design was repeated at two sites that represent different outcomes of sympatry: (1) a xeric mountain ridge where many hybrids are established (HZ); and (2) a mesic valley bottom where virtually no hybrids are established (MOCYN). By measuring fruit survival at several developmental stages, both the timing and strength of reproductive barriers within and between sites, species, cross types, and pollen dosage levels were examined. In three of four cases, heterospecific fruit set was significantly reduced compared to conspecific fruit set. This reduction occurred after the time of fertilization, but before the onset of embryo growth. Increasing the dose of pollen from an average of 9–194 grains/stigma did not affect this result. Thus, early postfertilization processes play a strong role in species fidelity in these oaks. *Quercus gambelii* experienced a five-fold decrease in conspecific fruit set at HZ relative to MOCYN. In contrast, heterospecific fruit set of *Q. gambelii* was the same at both sites. Poor *Q. gambelii* pollen performance is implicated as playing the major role in this result. One *Q. gambelii* individual at HZ was highly fecund, and had higher heterospecific than conspecific fruit set; slight introgression in this tree was detected using RAPD markers. The Environmental Emasculation Hypothesis that posits that environmental stress can increase the probability of hybrid formation by reducing the competitive ability of male gametes of one species is proposed.

Keywords: environmental emasculation hypothesis, hybrid zone, pollen competition, pollen quality, RAPD markers, reproductive barriers.

Introduction

Oaks represent a classic example of a taxonomic group in which individual species maintain distinct morphological, genetic and ecological identities despite extensive hybridization and introgression within the group (Stebbins, 1950; Burger, 1975; Grant, 1981; Whittemore & Schaal, 1991). Because oaks appear to have weak internal barriers to hybridization, it has long been argued that the degree to which hybrids appear is largely dependent on factors that control seedling establishment and survival (Muller, 1952; Stebbins *et al.*, 1947; Rushton, 1993). Oaks, along with other wind-pollinated

species, are seen as bearing the ‘cost’ of hybridization, producing many hybrid offspring that are destined either to fail or to be restricted to narrow or ephemeral habitats (Grant, 1958; Stebbins *et al.*, 1947; Hardin, 1975). As a result, oaks have been proposed as a model taxon for species concepts that rely on ecological criteria, rather than reproductive isolation, in delimiting species boundaries (Van Valen, 1976).

Ecological differentiation can act as a strong external barrier to gene flow. It can also influence internal reproductive barriers, although this possibility has generally been overlooked in the literature on oaks and on the formation and maintenance of hybrid zones (e.g. Anderson, 1948; Muller, 1952; Endler, 1977; Moore, 1977; Barton & Hewitt, 1985). For example, it has often been noted that when the ranges of two species overlap broadly, hybrids occur more frequently at the

*Correspondence and present address: Department of EPO Biology, University of Colorado, Boulder, CO 80309, USA. E-mail: joe.williams@colorado.edu

geographical or ecological margins of one or both species (Grant, 1981; Stebbins, 1950). Typically, this result is attributed to the presence of nonparental habitat types in such areas, habitat types in which hybrid survival is enhanced (Anderson, 1948; Moore, 1977). However, another possible explanation for the increased abundance of hybrids in such habitats is the breakdown of mate recognition systems due to the stress associated with existence at the margin of a species range. Few studies have examined reproductive processes that contribute to the formation of natural plant hybrid zones (Arnold, 1997).

The *Quercus gambelii* Nutt. \times *Q. grisea* Liebm. complex offers an opportunity to explore the role of internal reproductive barriers in modulating the occurrence and abundance of hybrids in zones of sympatry. These species exhibit geographical variation in the degree of hybridization (Tucker, 1961; R. Spellenberg, pers. comm.). In addition, there appears to be local variation; hybrid swarms are often found on xeric mountain slopes, but are rarely found in nearby mesic valleys. This study examines two such sites: (1) a site with a hybrid swarm containing a full range of intermediates; and (2) a contact zone with virtually no hybrid establishment. By comparing characteristics of individuals from sites with different levels of reproductive isolation, one may identify trait differences that account for the isolation. One may also gain insight into the processes contributing to the maintenance or loss of reproductive isolation. Specific traits studied at both sites were: (1) Comparative reproductive success of self, conspecific, and heterospecific experimental crosses; and (2) the relative timing of flower/fruit abortions from the time of fertilization to seed maturity. In addition, the effect of pollen dosage on flower/fruit survival was examined.

Materials and methods

The species

Quercus gambelii and *Q. grisea* are distantly related white oaks (subgenus *Quercus*; Nixon, 1993a). *Quercus gambelii* is most closely related to the mesic oaks of the *Q. margaretta* complex (Muller, 1952). *Quercus grisea* is most closely related to the xeric Mexican oaks (Nixon, 1993b). These two species are well separated in growth form, leaf anatomy, embryo structure, physiological tolerances and edaphic requirements. *Quercus gambelii* is commonly associated with the ponderosa pine belt of the south western U.S. mountains, whereas *Q. grisea* occurs at lower elevations as a codominant in the pinyon-juniper zone. The ranges of the two species overlap across a broad zone from northern Sonora/

Chihuahua, Mexico to southern Colorado (see Howard *et al.*, 1997). The hybrids between *Q. gambelii* and *Q. grisea* plus five other southwestern USA oak species were formerly recognized as the species *Q. undulata* Torr. (Tucker, 1961).

Study sites

The two study sites are located in the northern San Mateo Mountains, near Magdalena, New Mexico. This mountain range rises from the Chihuahuan Desert to the south and the central New Mexican volcanic plateau to the north, and endemic oak populations are effectively isolated from other large populations of oaks. The nearest congener, *Q. turbinella*, occurs at lower elevations, approximately 80 km south of the study area. One can, therefore, study variation in the level of genetic isolation between *Q. gambelii* and *Q. grisea* free from the confounding influence of other oak species.

The first site (Hybrid Zone, or HZ) lies at an elevation of 2676 m. This site contains both parental species and a full range of intermediates ('Mixed site' of Howard *et al.*, 1997). The area is dry and along the slopes of a mountain ridge. *Quercus grisea* is near its upper elevation limit here, while *Q. gambelii* is at its lower elevation limit (with the exception of riparian areas). Codominants at the site include a diverse array of shrubs and trees such as *Cercocarpus* spp., *Juniperus* spp., *Pinus edulis*, *P. flexilis*, *P. ponderosa*, and *Pseudotsuga menziesii*. The soils are rocky and shallow, with little grass cover between trees.

The second site (Monica Canyon, or MOCYN) is approximately 6 km north of the first site, in a spring-fed valley at an elevation of 2346 m. Here *Q. gambelii* is restricted to the deeper soils of the valley bottom, along with *Pinus ponderosa*, *Populus angustifolia*, *Pseudotsuga menziesii*, and *Salix* spp. *Quercus grisea* is largely restricted to the surrounding hillsides where it is a codominant with *Pinus edulis* and *Juniperus* spp. Morphological intermediates are conspicuously absent from this site.

At both sites, individuals of both species were common and relatively evenly distributed within their respective habitats. Contact zones were sharp transitional areas where both species often occurred within 1 m of each other. The contact zone at MOCYN is clearly delimited by a valley/hillside transition, whereas at HZ a more complex topography generally produced contact zones along north- and south-facing slopes. Individuals were relatively large and of single or few ramets at MOCYN and smaller and of multiple ramets at HZ. Both species flowered concurrently at the two sites (Williams & Boecklen, unpubl. data). Thus, the two sites do not differ in the degree of sympatry.

Selection of trees

In a study of phenology, we previously identified 10 reproductively mature trees of each species along parallel transects bordering a contact zone at each site (Williams & Boecklen, unpubl. data). Species were identified according to habitat preference, growth form and leaf morphology. As slope and aspect can be important determinants of microhabitat segregation in oaks (Benson *et al.*, 1967), an attempt was made to hold these constant within species. Four trees from each of these four transects were randomly selected for experimental pollinations. Conspecific individuals were > 40 m apart from each other, and there was no genetic evidence for clonal reproduction (Williams, unpubl. data). Voucher specimens of each of the 16 experimental trees were deposited in the New Mexico State University herbarium. Because hybridization clearly occurs at HZ, the possibility existed that the experimental trees were not 'pure' representatives of *Q. gambelii* and *Q. grisea*. To assess this possibility, we characterized the trees via randomly amplified polymorphic DNA (RAPD) markers.

RAPD analysis

In a survey of 700 10-bp primers, Howard *et al.* (1997) identified six primers that generated eight banding regions consistent with eight independently inherited genetic loci. These loci yielded 14 informative character states, or markers (the presence or absence of a particular fragment), that distinguished three geographically isolated *Q. gambelii* populations ($n=41$) from three geographically isolated *Q. grisea* populations ($n=20$). The same markers also distinguished the two species locally at sites above and below the zone of overlap (within which the present study was conducted). These primers were then used to characterize trees from the zone of overlap and demonstrated that character index scores based on RAPD genotypes correlated well with a morphological classification of the trees (Howard *et al.*, 1997).

The 16 chosen trees were characterised using the same six primers. A phenotype specific to *Q. grisea* was assigned a score of -1 . A phenotype specific to *Q. gambelii* was assigned a score of $+1$. The character index score of an individual represented the sum of its scores over all six primers. Because the six primers generated six *Q. grisea* markers and eight *Q. gambelii* markers, the score of an individual could range from -6 to $+8$. Two loci provided markers that were informative in one state but not in the alternative, thus a pure individual might not have the maximum score for that species. Mixed-species ancestry or introgression was

indicated by the presence of markers typical of both species in a single individual.

Experimental pollinations

Before bud swelling occurred, 60 shoots of the previous year (= 'branch') on each experimental tree were selected from south-facing portions at mid canopy height, although on smaller trees some branches were also from the upper canopy. At each site a full diallel cross (Wright, 1976), consisting of all possible reciprocal crosses among the 8 experimental trees was performed. Each cross was performed at three pollen dosage levels. Thus, each tree received three doses \times eight male donors (= 24 treatments), each replicated twice. Each of the 48 treatments was randomly assigned to a branch. Unused branches were self-pollinated as, due to the design of a diallel cross, self-crosses are poorly represented. Due to the unpredictability of flower production and slight losses due to wind and elk, several crosses were not fully replicated. This design allowed an assessment of male and female reproductive success as a function of site, species, cross type (self, conspecific or heterospecific), pollen dose and individual tree.

White oaks are wind-pollinated and monoecious. Male flowers, borne on dense inflorescences, or 'catkins', develop near the terminal end of the shoot of the previous year. Female flowers develop in the axils of the leaves from the shoot of current year. When bud swelling occurred in early May, male buds were stripped from branches, white paper bags (#6) were sealed around each branch, and the female flower-bearing shoots allowed to grow into the bags. All branches were bagged well before female flowers became receptive. Female flower receptivity lasts up to two weeks, while pollen shedding can last up to one week (Wright, 1976). Within an individual tree pollen shedding began slightly before the onset of female flower receptivity. Bags were removed from branches after all chance of natural pollination was over.

As no control was possible over the number of female flowers produced in each pollination bag, it can be regarded as a measure of female reproductive effort. At the time of pollination, the number of receptive female flowers (those with swollen ovaries and reflexed stigmas) within each bag was recorded. Flowers that did not reach this stage could not be reliably counted, and were visually estimated on a 'per tree' basis. As a measure of male reproductive effort, male catkin production over the entire tree crown at the time of anthesis was visually estimated and ranked (methods modified from Sharp & Chisman, 1961; Graves, 1980): 1 = virtually no catkins; 2 = only a few shoots with

catkins; 3 = a few catkins on most shoots; 4 = many catkins on almost all shoots.

Catkins approaching full elongation were collected from each tree and allowed to dry and dehisce in paper bags for up to 24 h. For most trees, catkins were collected on two or more occasions to ensure freshness of pollen for later pollinations (i.e. pollen always ≤ 2 days old). Pollen was stored in vials, and moisture content and temperature were controlled by placing the vials in a desiccator on ice. Pollen viability was not assessed, but experimental crosses using oak pollen, including these same two species, have demonstrated high pollen viability for over two weeks (Cottam *et al.*, 1982; Wright, 1976; Steinhoff, 1993). Pollinations were performed by removing a bag and pollinating all flowers with swollen ovaries and wet stigmatic surfaces. The flowers on each tree were pollinated after the peak of pollen shedding of that tree. This is the time period when stigmas are maximally receptive (Wright, 1976). Windy conditions were avoided during pollinations. No rain fell during the entire pollination period.

Each flower was pollinated with one of three dosage levels. A low dose was applied with a piece of fishing line (0.45 mm diameter), a medium dose was applied with a toothpick, and a high dose was applied with a plastic-filamented touch-up paintbrush. An attempt was made to place pollen on the same area of the stigmatic lobes in all pollinations; however, high doses were necessarily more extensive in coverage. All pollinations were performed by JHW. Pollen applications were practiced beforehand and the number of grains counted until a consistent dosage level was attained. Flowers were periodically checked to confirm application levels (mean (SE) number of pollen grains/stigma: low = 9.3 (1.3), medium = 30.4 (4.4), high = 194.2 (21.8)). These doses are within the range of natural pollen loads (Williams & Boecklen, unpubl. data).

Flower and fruit survival

Flower or fruit abortion is episodic in oaks and occurs during several distinct developmental stages (Table 1): during floral development (pollination period), during ovule development and pollen tube growth (period 1), and during formation of the endosperm and early embryo (period 2; Feret *et al.*, 1982; Sork *et al.*, 1993). After embryo growth begins (period 3), abortion is negligible and is often associated with herbivore damage (Williamson, 1966). A final period of maturation ends when the acorn falls from the tree (period 4). Differentiation between prezygotic (period 1) and postzygotic (periods 2–4) phenomena were determined by monitoring the success of crosses from pollination to maturity. In order to best represent the abortion peaks occurring within each developmental period, the number of surviving flowers/fruits was counted one week after the end of each of these developmental periods were counted (sampling points (SP) 1–4; Table 1). Fruits were considered to be aborted if they had fallen, if they had brown stalks indicative of impending abscission, and/or if they displayed browning at the base of the style or apex of the cupule (Sharp & Sprague, 1967). For statistical analyses, fruit survival at SP1, SP2 and SP3 was calculated as the number of nonaborted fruits remaining divided by the number of flowers pollinated. At SP4, survival (fruit set) was calculated as the number of nonaborted acorns that fell into protective screens, divided by the number of flowers pollinated. Non-abortive acorns were characterized by embryo swelling extending above the cupule rim.

Statistical analysis

The experimental pollinations followed a full diallel cross at each site. However, the standard analysis for

Table 1 Timeline of developmental periods during white oak reproductive cycle (from Turkel *et al.*, 1955; Stairs, 1964; Sharp & Sprague, 1967; Mogensen, 1975; not to scale). Ovule formation begins after pollination, with the embryo sac being ready for fertilization 4–5 weeks later. After fertilization, endosperm development occurs first, with cell division of the zygote beginning 1–2 weeks later, there is little visible growth of the fruit during period 2. Flower/fruit survival from experimental pollinations was monitored 6 weeks, 10 weeks and 14 weeks after pollination (SP1, SP2, SP3); and at the end of mature fruit fall (SP4)

Pollination 10–14 days	Period 1 4–5 weeks	Period 2 4 weeks	Period 3 4 weeks	Period 4 4–8 weeks
pollination and pollen germination	pollen tube growth, ovule formation and fertilization	cell division of endosperm followed by differentiation of early embryo	embryo (acorn) enlargement	acorn ripening and drop
late May to early June	June to early July	July to early August	August to early September	September to October

this design (see Lynch & Walsh, 1998) was not used because we were not interested in estimating traditional variance components, such as those for sires and dams. Instead, the main interest was in differences in patterns of reproductive success in two different ecological settings. Thus, a nested, mixed-model ANOVA was used with site, species, cross-type, and pollen dose as fixed effects; trees nested within site and species, and branches nested within trees (error term), as random effects. Hypothesis tests were based on maximum likelihood ratio tests, which approximate a chi-squared (χ^2) distribution (Dixon *et al.*, 1990). Models were constructed to examine variation in female and male function separately. The response variable for female function was percent fruit survival/treatment at SP1 and at SP4. The components of female function were analysed separately. In addition, male function was examined using the response variable of percent fruit survival/treatment at SP4 according to pollen donor trees.

Results

RAPD analysis

Character index scores of *Q. grisea* from allopatric populations range from -6 to -2 with a mean of -5.4 (Howard *et al.*, 1997). Scores of *Q. gambelii* from allopatric populations range from +5 to +8 with a mean of +7.1. The character index scores of the 16

study trees are listed in Table 2. Most of the *Q. gambelii* and *Q. grisea* individuals had scores within the range found among allopatric representatives of the species. The exceptions were *Q. gambelii* tree #2, that had a score of +4 and *Q. grisea* tree #16, that had a score of 0. Six of the eight *Q. gambelii* trees and all of the *Q. grisea* trees displayed at least one RAPD character (a marker or the lack of a marker) typical of the other species (Table 2).

Reproductive effort

Male reproductive effort, as measured by catkin production, was consistently high in *Q. grisea* (Table 3). *Q. gambelii* was more variable, with some trees at HZ producing virtually no catkins. These same trees were among the highest female flower producers (Table 3). At HZ, the pattern of high pollen production by *Q. grisea* and low pollen production by *Q. gambelii* was also reflected in a larger sample of trees (Williams & Boecklen, unpubl. data).

Many *Q. grisea* flowers were aborted prior to maturation, and as a result, *Q. gambelii* produced 1.86 times as many receptive flowers as *Q. grisea* (Table 3).

Fruit survival and maturation

Fruit survival at the end of period 1 (SP1) differed significantly between species (Table 4). *Quercus gambelii*

Species and location	Tree	Score	# markers conspecific	# markers heterospecific
<i>Quercus gambelii</i>				
Hybrid zone	1	+ 6	7	1
	2	+ 4	6	2
	3	+ 6	7	1
	4	+ 6	7	1
	9	+ 6	7	1
Monica Canyon	10	+ 7	7	0
	11	+ 6	6	0
	12	+ 6	7	1
Mean		+ 5.88		
<i>Quercus grisea</i>				
Hybrid zone	5	- 2	4	2
	6	- 2	4	2
	7	- 2	4	2
	8	- 2	4	2
Monica Canyon	13	- 2	4	2
	14	- 2	4	2
	15	- 4	5	1
	16	0	3	3
Mean		- 2.00		

Table 2 Character index scores based on RAPD markers in *Quercus gambelii* and *Q. grisea*. Scores can range from -6 (*Q. grisea*) to +8 (*Q. gambelii*)

Table 3 Male and female reproductive effort in *Quercus gambelii* and *Q. grisea*. Abscission = relative estimate of the amount of female flower abscission observed during hand pollinations (i.e. flowers that failed to complete development and could not be pollinated); flowers = the total number of receptive flowers pollinated; *n* = the total number of pollination bags included in the analysis; catkins = estimated male inflorescence production ranked on a scale of 0–4

Species and location	Tree	Abscission	Flowers	<i>n</i>	Catkins	
<i>Quercus gambelii</i>						
Hybrid zone Monica Canyon	1	None	397	50	1	
	2	None	604	45	2	
	3	Low	504	49	0	
	4	None	149	46	0	
	9	High	69	23	0	
	10	None	150	39	4	
	11	None	258	45	4	
	12	None	648	51	4	
	Total			2779	348	
	<i>Quercus grisea</i>					
	Hybrid zone Monica Canyon	5	None	269	52	4
		6	Moderate	349	48	4
7		Moderate	82	39	4	
8		High	105	37	4	
13		None	235	46	3	
14		Moderate	202	43	3	
15		High	75	38	4	
16		Low	177	48	4	
Total			1494	351		

Table 4 ANOVA results (maximum likelihood method) measuring fertilization success in terms of female function (% fruit survival at SP1) in *Quercus gambelii* and *Q. grisea*

Effect	d.f.	Chi-square	<i>P</i>
Site	1	0.054	0.815
Species	1	14.413	< 0.001
Cross	2	0.926	0.629
Dose	2	0.593	0.743
Site × Cross	2	0.661	0.719
Site × Dose	2	1.688	0.430
Site × Species	1	0.132	0.716
Species × Cross	2	14.588	0.001
Species × Dose	2	0.531	0.531
Cross × Dose	4	0.625	0.960
Site × Species × Cross	2	0.315	0.315
Trees (Site, Species)	1	72.811	< 0.001

had lower overall fruit survival during this time period than did *Q. grisea* (Fig. 1). In both species, fruits resulting from conspecific and heterospecific fertilizations survived with equal frequency (Fig. 1). In contrast, self-crosses survived differently than nonself crosses. In *Q. gambelii*, fewer self than nonself crosses survived to SP1. In *Q. grisea* more self than nonself-crosses survived to SP2 (Fig. 1). The significance of this difference is confirmed by the high χ^2 value of the species × cross interaction (Table 4).

The final factor to have a significant effect on fruit survival during period 1 was trees (Table 4). This finding indicates there was variation in fruit survival among

individual trees. Site and dose did not influence fruit survival, nor were any other interactions significant (Table 4).

Fruit survival measured at SP2 was virtually identical to fruit survival at SP3 and SP4 (i.e. fruit set). Only 3.5% of all crosses aborted during the 3 months between SP2 and SP4. Therefore, rather than evaluating fruit survival at SP2, 3 and 4 separately, only the data from SP4 is reported, recognizing that these results occurred during period 2.

In contrast to SP1, species effects were nonsignificant at SP4 (Fig. 2; Table 5). Total fruit set was equivalent in both species (mean (\pm SE) fruit set for *Q. gambelii* = 0.121 (0.015), for *Q. grisea* = 0.127 (0.018)). The effect of dose, both as a main effect and in all interactions, was also nonsignificant (Table 5).

Cross type was significant both as a main effect and in all interactions other than dose (Fig. 2; Table 5). In general, conspecific outcrosses were more successful than self- and heterospecific crosses. However, the significant site × species × cross interaction highlights a dramatic reduction in conspecific outcrossing success for *Q. gambelii* at HZ. *Quercus gambelii* conspecific fruit set dropped almost five-fold from MOCYN to HZ ($\chi^2 = 5.42$, $P = 0.02$, d.f. = 1; Fig. 2). In contrast, *Q. gambelii* heterospecific fruit set did not differ between MOCYN and HZ ($\chi^2 = 1.04$, $P = 0.31$, d.f. = 1; Fig. 2). Thus, when *Q. gambelii* pollen was applied to *Q. gambelii* stigmas fruit set was poor, whereas when *Q. grisea* pollen was applied, fruit set was relatively high. This pattern of variation suggests that *Q. gambelii*

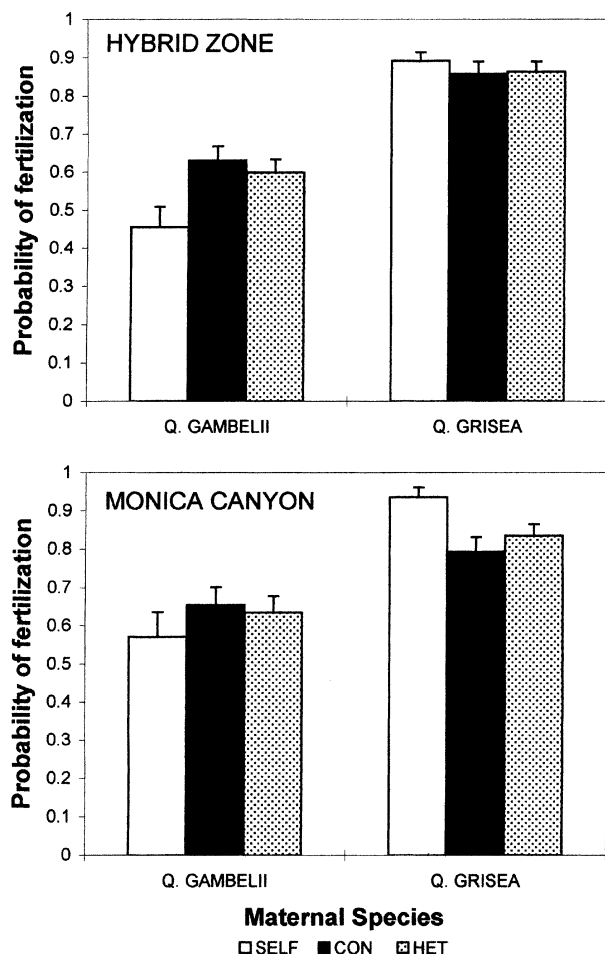


Fig. 1 Percent fruit survival 6 weeks after pollination (SP1). CON = conspecific; HET = heterospecific. Error bar = 1 S.E.

females at HZ were fertile and that the reduction in their conspecific outcrossing success was largely attributable to poor *Q. gambelii* pollen performance.

The reproductive success (RS) of *Q. gambelii* males at HZ was lower than the RS of all other groups of males. The significance of this difference is confirmed by a male function, site \times species interaction effect (Table 6). At HZ, *Q. gambelii* pollen performed poorly regardless of the species to which it was applied.

Discussion

Variation in reproductive success in *Quercus gambelii*

Quercus grisea had equivalent reproductive success at both sites for each cross type. In contrast, the conspecific cross type of *Q. gambelii* was significantly less successful at HZ than MOCYN. One possible explanation for this outcome is stress. *Quercus gambelii*

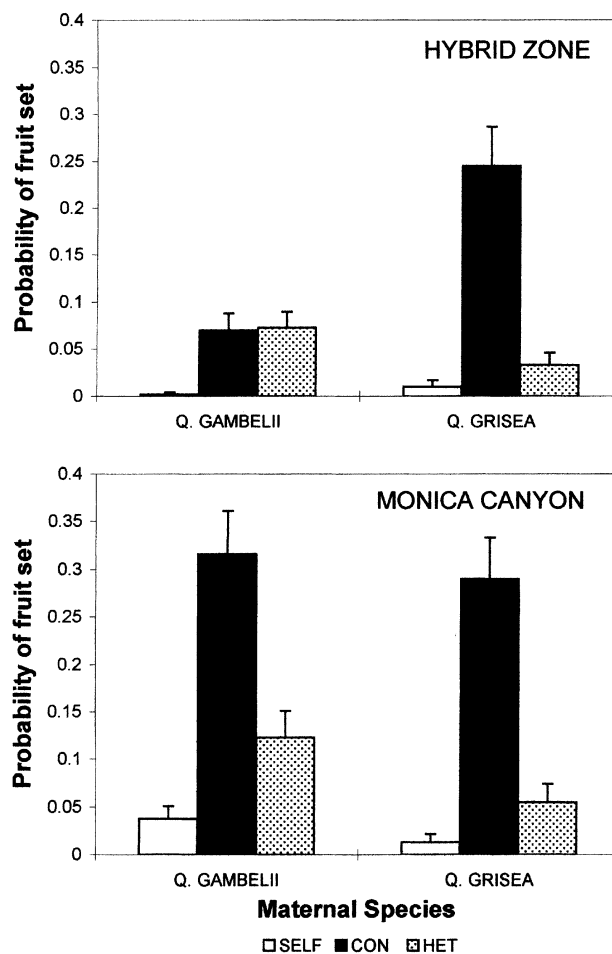


Fig. 2 Percent fruits matured (SP4). CON = conspecific; HET = heterospecific. Error bar = 1 S.E.

Table 5 ANOVA results (maximum likelihood method) measuring fruit set in terms of female function (% fruits matured at SP4) in *Quercus gambelii* and *Q. grisea*

Effect	d.f.	Chi-square	P
Site	1	2.926	0.087
Species	1	0.022	0.882
Cross	2	119.857	< 0.001
Dose	2	2.612	0.271
Site \times Cross	2	11.188	0.004
Site \times Dose	2	1.315	0.518
Site \times Species	1	1.134	0.287
Species \times Cross	2	15.979	< 0.001
Species \times Dose	2	1.465	0.481
Cross \times Dose	4	1.657	0.798
Site \times Species \times Cross	2	7.932	0.019
Trees (Site, Species)	1	52.798	< 0.001

Table 6 ANOVA results (maximum likelihood method) measuring fruit set in terms of male function (% fruits matured at SP4) in *Quercus gambelii* and *Q. grisea*

Effect	d.f.	Chi-square	P
Site	1	8.279	0.004
Species	1	0.067	0.796
Cross	2	98.716	< 0.001
Dose	2	2.913	0.233
Site × Cross	2	10.465	0.005
Site × Dose	2	1.145	0.564
Site × Species	1	4.310	0.038
Species × Cross	2	8.835	0.012
Species × Dose	2	1.395	0.498
Cross × Dose	4	1.233	0.873
Site × Species × Cross	2	5.349	0.069
Trees (Site, Species)	1	3.531	0.060

is a northerly distributed, mesic habitat species, that may be under considerable stress at the HZ site. At this site, representatives of *Q. gambelii* occur on dry, inorganic hillside soils. In contrast, at the MOCYN site, *Q. gambelii* occurs on a valley floor that has richer, wetter soils. It is likely that *Q. grisea* is under less stress at HZ than *Q. gambelii*, because at both sites it occurs on dry hillsides, a fairly typical habitat for the species.

Environmental stress can affect sexual reproduction through a reduction of male or female function, or both (Freeman *et al.*, 1981; Bertin, 1988; Young & Stanton, 1990; Stephenson *et al.*, 1993; Delph *et al.*, 1997; Saini, 1997). For example, Aizen & Raffaele (1998) report significant reduction in pollen quality in *Alstroemeria aurea* following artificial defoliation, while Lau & Stephenson (1993, 1994) document reduced pollen quality and siring success in *Cucurbita pepo* grown in either nitrogen or phosphorus poor soils. The results reported here suggest that a reduction in male function is responsible for the poor reproductive success of conspecific pollinations of HZ *Q. gambelii*. Female function is less implicated as a factor in this result because heterospecific fruit set of HZ *Q. gambelii* was not significantly reduced relative to that of MOCYN *Q. gambelii*. Moreover, male RS was reduced in HZ *Q. gambelii* regardless of cross type. Another indication of poor male function was the very low pollen production of HZ *Q. gambelii*. Low pollen production can be associated with low pollen vigour (Schlichting, 1986; Schoper *et al.*, 1986; but see Cruzan, 1990). Female flower production, conversely, was not low in *Q. gambelii* at HZ (Table 3). Thus, factors associated with male performance appear to play a stronger role than those associated with female performance in

causing low conspecific outcrossing success of HZ *Q. gambelii*.

In light of this conclusion, it might be argued that the *Q. gambelii* trees at HZ are introgressed, which caused a reduction in pollen viability or vigour. This argument is not supported by the RAPD analysis. The RAPD profiles of the four *Q. gambelii* trees at HZ were similar to the profiles of the trees at MOCYN (Table 2). Moreover, *Q. gambelii* tree #2 with the lowest character index score at HZ, hence, the most highly introgressed tree, produced considerably more pollen than the other trees at HZ. In addition, the pollen of this tree had nonzero siring success on all other females, conspecific or heterospecific. Finally, the lack of a pollen dose effect suggests that reduced male function was not due to a reduction in the number of viable pollen grains produced, which might be expected if introgression had led to disturbed meiosis. Thus, introgression does not appear to explain the reduction in male function observed among *Q. gambelii* at HZ.

Environmental Emasculation Hypothesis

The results reported here are consistent with the hypothesis that the formation of hybrid zones in the *Quercus gambelii* × *Q. grisea* species complex is a consequence of diminished male function in *Q. gambelii* owing to environmental stress. One effect of decreased male function in *Q. gambelii* would be an increase in hybrid fruit set relative to conspecific fruit set. Thus, one explanation for the increased frequency of hybrids at the HZ site is poor pollen quality in *Q. gambelii*. Poor pollen quality also reduces pollen competitive ability, and pollen competition can be an important arbiter of species boundaries in other wind-pollinated tree species (Williams *et al.*, 1999). An obvious, but untested, prediction of this hypothesis is that most of the hybrids at this site should harbour *Q. gambelii* mitochondrial and chloroplast DNA.

Asymmetric patterns of introgression appear to be common in both European and American oaks (Whittemore & Schaal, 1991; Ferris *et al.*, 1993; Bacilieri *et al.*, 1996; Dumolin-Lapègue *et al.*, 1997; Howard *et al.*, 1997). There is also evidence of asymmetric cross-compatibility between oak species (Aas, 1991; Steinhoff, 1993). At least one study has sought to explain such patterns through a combination of ecological, demographic and reproductive factors, including male function (Petit *et al.*, 1997). This study offers a new explanation for both variation in hybrid zone formation as well as for why such zones often display asymmetric patterns of hybridization, although these data cannot be viewed as a test of the environmental emasculation hypothesis. A test of the hypothesis would require zones

of sympatry (both with and without significant hybridization) to be the experimental units, and the present experimental design had no replication at the level of zones of sympatry.

Some models of hybrid zone dynamics assume that hybrids are inherently less fit than their parents (Endler, 1977; Barton, 1979; Barton & Hewitt, 1985), while other models assume that hybrids have reduced fitness in parental environments but increased fitness in novel or intermediate environments (Anderson, 1948; Moore, 1977). Arnold & Hodges (1995) have argued that reduced hybrid fitness measured in many studies is often an average across a heterogeneous array of genotypic classes. The discovery that the most successful producer of pollen and fruits among the *Q. gambelii* at the HZ site (tree #2) appeared to be an advanced backcross, represents further evidence that some hybrids are not necessarily less fit than their parents, particularly in marginal habitats.

Flower and fruit abortion

Many long-lived perennials, including oaks, produce far more flowers than mature fruits (Stephenson, 1981). Three periods of heavy flower/fruit abortion were recorded. Both species had substantial flower abortion prior to fertilization (*Q. grisea* before pollination, *Q. gambelii* after pollination). Importantly, these abortions were random with respect to cross type, and suggest an early maternal adjustment to resource availability (Lloyd, 1980).

Mate discrimination in white oaks appears to be a consequence of postzygotic processes. Self-pollinated flowers aborted at the same rate as conspecific and heterospecific flowers before fertilization (period 1), but were nearly completely eliminated after fertilization (during period 2). In addition, the low heterospecific RS seen in both species also occurred during period 2, as well as the dramatic reduction in *Q. gambelii* conspecific RS at HZ (compare Figs 1 and 2). Almost no abortions occurred during acorn growth and maturation (periods 3 and 4). This pattern suggests mate recognition occurs via postzygotic mechanisms, such as late-acting incompatibility and inbreeding or outbreeding depression, but not by prezygotic self or heterospecific incompatibility.

The abortion peak late in period 2 corresponds to the period when the early embryo first undergoes differentiation. Wiens *et al.* (1987) suggest that a heavy clustering of developmental lethals at this time is the major cause of conspecific embryo abortion in outcrossing species. Poor pollen quality may also lead to increased embryo abortion. For example, slower pollen tube growth may result in delayed fertilization, leading to developmental selection amongst embryos (Buchholz,

1922). It has been shown in other flowering plants, such as *Phaseolus* spp., that seeds with late-forming endosperm and embryos have a higher likelihood of abortion (Rabakoarihanta *et al.*, 1979; Nakamura, 1986).

An alternative interpretation of the high abortion rate in period 2 is that these abortions represent unfertilized flowers which aborted late. This interpretation seems unlikely, for several reasons. First, ample time was allowed for crosses that could not fertilize eggs to express signs of abortion. In fact, during period 1, discrimination between self and nonself was expressed through differential flower abortion (Fig. 1). Second, almost all abortions recorded at SP1 were flowers that had already fallen, rather than flowers that were turning brown (J. H. Williams, pers. obs.). This indicates that SP1 occurred well after an earlier abortion peak. In contrast, at SP2 many aborted fruits were still on the tree (i.e. fruits turning brown and/or initiating an abscission layer). This indicates that the second abortion peak occurred late in period 2. Thus, if the abortions which occurred during period 2 were due to lack of fertilization, the delay of any sign of impending abortion was approximately 4–6 weeks long. It appears that both the reduced heterospecific RS and the reduced conspecific RS of *Q. gambelii* at HZ were due to an increase in embryo abortion rather than to a decrease in the ability of pollen tubes to fertilize ovules.

Pollen dosage experiment

Heterospecific pollen swamping of isolated plants has been implicated in the production of hybrids in nature (e.g. Arnold *et al.*, 1993; Tucker, 1963; Williams *et al.*, 1999). If variation exists for pollen tube growth rate or vigour, then increasing the amount of heterospecific pollen on a stigma might be expected to increase the probability of fertilization. In fact, neither conspecific or heterospecific fertilization increased when pollen loads were increased from approximately 9–194 pollen grains. This is further evidence for a lack of strong pre-fertilization barriers in these species, at least when heterospecific pollen occurs alone on a stigma. Whether conspecific pollen outcompetes heterospecific pollen for fertilizations when both types of pollen occur on a stigma awaits further study.

Conclusions

In oaks and many other groups of species, the level of reproductive isolation between closely related species varies geographically. By comparing characteristics of individuals from areas with different levels of reproductive isolation it becomes possible to pinpoint factors that mediate reproductive isolation. The results of this study

indicate that marginal habitats can increase the probability of hybrid offspring formation by altering the quantity and quality of male gametes (environmental emasculation) of one parental species.

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