Pollen competition as a unilateral reproductive barrier between sympatric diploid and tetraploid *Chamerion angustifolium*

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Speciation requires the evolution of barriers to gene exchange between descendant and progenitor populations. Cryptic reproductive barriers in plants arise after pollination but before fertilization as a result of pollen competition and interactions between male gametophytes and female reproductive tissues. We tested for such gametic isolation between the polyploid *Chamerion angustifolium* and its diploid progenitor by conducting single (diploid or tetraploid) and mixed ploidy (1 : 1 diploid and tetraploid) pollinations on both cytotypes and inferring siring success from paternity analysis and pollen-tube counts. In mixed pollinations, polyploids sired most (79%) of their own seeds as well as those of diploids (61%) (correcting for triploid block, siring success was 70% and 83%, respectively). In single donor pollinations, pollen tubes from tetraploids were more numerous than those from diploids at four different positions in each style and for both diploid and tetraploid pollen recipients. The lack of a pollen donor $\times$ recipient interaction indicates that the tetraploid siring advantage is a result of pollen competition rather than pollen–pistil interactions. Such unilateral pollen precedence results in an asymmetrical pattern of isolation, with tetraploids experiencing less gene flow than diploids. It also enhances tetraploid establishment in sympatric populations, by maximizing tetraploid success and simultaneously diminishing that of diploids through the production of inviable triploid offspring.

Keywords: flow cytometry; hybridization; prezygotic barrier; reproductive isolation; speciation

1. INTRODUCTION

Species formation requires the evolution of barriers to gene exchange between progenitor and descendant populations, and the promotion of conspecific rather than heterospecific matings. These barriers may arise through a variety of mechanisms by preventing fertilization or hybrid formation. Post-mating isolation is manifested as low viability or fertility of hybrids, or ecological differentiation between hybrids and their parents (Schluter 1994). Pre-mating isolation may arise due to ecogeographical or behavioural differences, or physical incompatibilities between taxa. Pre-mating barriers are particularly important for the persistence of incipient species in sympatry because they prevent hybrid fertilizations and avoid reduced fitness of one or both taxa due to the wastage of ovules and sperm that would otherwise have been used on conspecific matings (Levin 1975).

Recently, it has been suggested that reproductive barriers acting after pollination or insemination but before fertilization may play an important part in limiting gene flow (Howard *et al.* 1998). Studies of pollen or sperm

competition have found evidence for conspecific gamete precedence (Smith 1968; Arnold *et al.* 1993; Howard & Gregory 1993; Riesberg *et al.* 1995) and indicate that interactions between male gametophytes (pollen) or sperm and the female reproductive tract can evolve quickly and may represent a primary barrier between closely related taxa. Furthermore, this mechanism of sexual isolation may be asymmetrical, such that the gametic interactions depend on the directions of mating (Arnold *et al.* 1996; Riesberg *et al.* 1995). Such interactions create asymmetries in the proportions of ovules (eggs) usurped by hybrid matings (Levin 1975) and hence in the direction of gene flow between the parental taxa.

We investigated the role of gametic barriers operating between diploid and tetraploid fireweed (*Chamerion angustifolium*) to understand the factors regulating the dynamics of these cytotypes in sympatry. Diploid and tetraploid fireweed are generally found at different latitudes but also co-occur in a broad contact zone extending along the southern limits of the boreal forest and on a north–south axis in the Rocky Mountains. Mixed populations of diploid and tetraploid fireweed are common in the contact zone (Husband & Schemske 1998; B. C. Husband, unpublished data). Diploid plants flower earlier than tetraploids, but 50% of all individuals can potentially receive heterospecific pollen. Although pollinator fidelity further reduces the opportunities for hybrid matings, bumble-bee pollinators frequently move between cytotypes (Husband & Schemske 2000) and, because each flower receives multiple visits, many stigmas will receive

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pollen from both cytotypes. The interactions between diploid and tetraploid pollen on plant stigmas and the potential for gamete selection are unknown for any species of mixed ploidy. In this study, we asked (i) does pollen from diploid and tetraploid plants have different siring abilities when applied together?; (ii) is siring success dependent on the ploidy of the pollen recipient?; and (iii) is the relative siring success of diploids and tetraploids related to pollen tube growth rates?

2. METHODS

(a) Source of parent material

Experimental crosses were performed on plants originating from separate seed families in two neighbouring populations, one diploid (population D2) and the other tetraploid (population T26), located along the Beartooth Highway in southern Montana and northern Wyoming, USA (Flint 1980; Husband & Schemske 1998). These populations occur within the diploid–tetraploid contact zone for this species (Mosquin 1966).

To compare the pollen production of the two cytotypes, pollen grain number per anther and pollen viability were measured in a random sample of 10 plants from each population grown in the greenhouse. Pollen production was estimated for two anthers from each plant; anthers were placed in 1 ml 70% ethanol and, after vortexing, three 0.1 ml subsamples were removed, mounted on a slide and the number of grains censused on a light microscope. For pollen viability, the contents of two anthers were placed in a drop of lactophenol blue on a microscope slide. After 15 minutes of incubation, the first 300 pollen grains encountered along arbitrary transects on the slide were scored as viable or inviable. Viable grains had a continuous cytoplasm that stained blue. Non-viable grains were empty or had a discontinuous cytoplasm and staining was incomplete or absent. Replicate values of viability and pollen production were averaged for each plant, and means for diploids and tetraploids were compared using a one-way analysis of variance (ANOVA).

(b) Offspring number

Seeds from three maternal plants of each cytotype (diploid or tetraploid) were germinated and grown in the University of Guelph greenhouses. Prior to flowering, a mesh bag was placed over each inflorescence to prevent pollinator visits.

Four hand-pollination treatments were performed on the flowers of each plant: (i) within-cytotype; (ii) between-cytotype; (iii) mixed cytotype; and (iv) control. The within-cytotype treatment consisted of pollen from four anthers of each of two male parents of the same cytotype. Between-cytotype pollinations comprised two male parents randomly selected from the three plants of the alternate cytotype. Mixed cytotype pollinations consisted of pollen (four anthers) from one diploid and one tetraploid parent. For each pollination treatment, we avoided giving either male parent an initial siring advantage by (i) randomly selecting the male parent whose pollen was to be applied first; (ii) applying pollen from each parent along opposite sides of each of the four stigma lobes (one anther per stigma lobe); and (iii) applying two anthers of pollen from male parent one, followed by four anthers of male parent two, followed by the final two anthers of male parent one. All pollen recipients were first emasculated to avoid self-fertilization. The control pollination comprised emasculating the flowers but applying no pollen, to check for inadvertent pollen transfer. Pollinations were

performed over a four-day period, each plant receiving all pollination treatments on each day.

Fruit were harvested two weeks later and seed production and the proportion of seed set estimated for each. Both response variables were analysed using an ANOVA, with maternal cytotype (diploid, tetraploid) as the whole-plot effect and paternal cytotype (diploid, tetraploid, mixed) as the split-plot effect.

(c) Offspring cytotype and siring rate

Offspring cytotype was derived from estimates of nuclear DNA content, which were generated using flow cytometry (Burton & Husband 1999; Husband & Schemske 2000). Within-cytotype pollinations ($2x \times 2x$, $n = 41$; $4x \times 4x$, $n = 43$) were used as controls to establish the expected range of DNA content for diploid and tetraploid offspring. Although diploid plants may occasionally produce gametes with more than the haploid complement of chromosomes, and thus triploids may periodically arise from $2x \times 2x$ crosses, Burton & Husband (2001) found this to be rare in *C. angustifolium* (less than 3%) and thus unlikely to influence results. Between-cytotype crosses ($2x \times 4x$, $n = 66$) were used to establish the expected range of DNA content for triploids. These ranges have been described previously (Husband & Schemske 2000). Seed from the mixed pollinations on diploid ($n = 62$) and tetraploid ($n = 117$) mothers were then screened and assigned to a cytotype (diploid, triploid or tetraploid).

The siring success of diploid and tetraploid pollen in the mixed pollination treatment was inferred from the offspring cytotype frequencies. Diploid and triploid offspring produced by diploid mothers were interpreted as being sired by diploid and tetraploid pollen, respectively. Similarly, triploid and tetraploid offspring from tetraploid mothers were interpreted as being sired by diploid and tetraploid pollen, respectively. We tested for heterogeneity in the frequencies of diploid- and tetraploid-sired seeds using a 2×2 contingency analysis (*G*-test).

The measured siring rates probably deviate from the actual siring patterns at the time of fertilization due to differential mortality of embryos formed by within- and between-cytotype crosses. We estimated the frequency of offspring fertilized by between-cytotype pollen using the equation $b = b_s/[w_b + b_s(1 - w_b)]$, where b is the rate of between-cytotype fertilization, b_s is the rate of between-cytotype mating measured at the seedling stage and w_b is the relative fitness of between-cytotype crosses for the stages of seed maturation and germination combined. Fitness estimates were taken from Burton & Husband (2000). To calculate b for diploid pollen recipients, the fitness measure for triploids was based on the results of between-cytotype crosses, with tetraploids as the male parent expressed relative to the fitness of diploids ($b_s = 0.31$). For tetraploid recipients, triploid fitness was based on between-cytotype crosses with diploids as the male parent and expressed relative to tetraploids ($b_s = 0.63$).

(d) Pollen-tube growth

Crosses were performed on five tetraploid and four diploid plants over a 10-day period. Each plant served as both a pollen donor and recipient. To serve as recipient on a given day, a plant had to have a minimum of two mature flowers and at least one eligible donor of each cytotype available. Plants with more than two open flowers received replicate pollinations as available, where each replicate involved a different pollen donor. In total, 139 crosses were conducted and each plant was pollinated by at least two diploid and two tetraploid donors.

Table 1. Summary of ANOVA of seed number and proportion of seed set in pollinations of diploid and tetraploid *Chamerion angustifolium*. Maternal plant (cytotype) was designated as random and used to test the effect of the maternal cytotype: all other sources of variation were treated as fixed effects. MS, mean squares.

source of variation	d.f.	MS	F
seed number			
maternal cytotype	1	3665.0	0.88
maternal plant (cytotype)	2	4168.8	8.97**
pollination treatment	3	20 969.0	45.09***
maternal cytotype $\times$ pollination treatment	3	19 433.0	41.79***
seed set			
maternal cytotype	1	0.013	0.32
maternal plant (cytotype)	2	0.039	4.56*
pollination treatment	3	0.176	20.41***
maternal cytotype $\times$ pollination treatment	3	0.151	17.50***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

We counted pollen tubes by staining them with aniline blue (modified from Martin (1959)). Styles were collected 6 h after pollination, fixed for 24 h (70 : 30 ethanol to acetic acid), cleared in 10 N NaOH for 24 h and stained with 0.1% aniline blue in 33 mM K_3PO_4 (Martin 1959). Styles were mounted in glycerin on a microscope slide and viewed under ultraviolet light on a fluorescent microscope. Pollen tubes were counted at three distances from the stigma (6 mm, 8 mm and 10 mm) and at the style base.

Variation in pollen-tube number was quantified using a two-factor ANOVA with nesting. The main effects were as follows: maternal ploidy, maternal plant (ploidy) (random effect), paternal ploidy, paternal plant (ploidy) (random effect), and the maternal $\times$ paternal cytotype interaction. The maternal plant and paternal plant effects, which were both nested, were used as the sources of error for the maternal and paternal ploidy effects, respectively. In some cases, especially at the 6 mm position in the style, pollen tubes were too numerous to count. For the statistical analyses, these measurements were given the value 50, which was the maximum number that could be counted with reliability. This analysis treats pollen-tube number as a continuous variable; the residuals were normal for all variables except at the 10 mm position (Shapiro Wilks $W = 0.95$, $p < 0.001$).

3. RESULTS

(a) Pollen number and viability

Diploid and tetraploid plants did not differ with respect to pollen production per anther (mean per subsample: diploid = 152.7, s.e. = 11.88, $n = 10$; tetraploid = 140.87, s.e. = 10.05, $n = 10$; $F = 0.57$, $p > 0.45$) or percentage pollen viability (mean percentage viable pollen: diploid = 90.7, s.e. = 0.026, $n = 10$; tetraploid = 91.2, s.e. = 0.028, $n = 10$; $F = 0.017$, $p > 0.75$).

(b) Seed production

No differences in mean seed number or percentage seed set were observed between diploid and tetraploid plants; however, the effect of pollination treatment and the pollination treatment by cytotype interaction were both significant (table 1). Mean seed number and the percentage seed set were highest in within-cytotype crosses (seed number: $2x = 230.7$; $4x = 134.8$; seed set: $2x = 59.3\%$,

Figure 1. Mean ($\pm$ s.e.) percentage seed set for diploid (open squares) and tetraploid (filled squares) *Chamerion angustifolium* when pollinated with pollen from diploids, tetraploids, or equal mixtures of both.

$4x = 46.3\%$; figure 1). Seed production decreased in both diploids and tetraploids as the proportion of pollen from the alternative ploidy increased. However, the percentage reduction in seed set was larger in diploids (83%) than in tetraploids (57%).

(c) Offspring cytotype and siring rates

Based on the premise that between-cytotype fertilizations yield triploids, while within-cytotype fertilizations produce diploids or tetraploids, we inferred the siring success of diploid and tetraploid pollen in mixed pollinations. Mixed pollinations were heterogeneous in the frequency of diploid- and tetraploid-sired seeds (2×2 contingency analysis, $G = 6.0$, $p < 0.02$). Overall, pollen from diploid plants sired 39% of seeds on diploid maternal plants and 21% on tetraploids (figure 2). Likewise, tetraploid pollen sired a greater percentage of seeds on tetraploid plants (79%) than on diploids (61%). However, for any given maternal cytotype (diploid or tetraploid), pollen from tetraploids sired more seed than pollen from diploids

Figure 2. Percentage of diploid (2 $\times$), triploid (3 $\times$) and tetraploid (4 $\times$) progeny produced in mixed pollinations (1 : 1) of diploid and tetraploid *Chamerion angustifolium*. Values are given for diploid and tetraploid maternal plants. The frequency of triploid offspring is used to infer the frequency of between-cytotype fertilizations.

(figure 2). Tetraploid pollen sired 61% of all seeds on diploid mothers ($n = 62$) and 79% on tetraploid mothers ($n = 117$). Correcting for differential mortality of conspecific and heterospecific zygotes prior to the seedling stage, pollen from tetraploids sired 83% of all seeds on diploids and 70% on tetraploids.

(d) Pollen tubes

Mean pollen-tube number in the style at 6 mm, 8 mm and 10 mm from the stigma and at the style base averaged 39.7, 34.7, 29.6 and 20.6, respectively. All analyses of variance were consistent in finding a significant effect of paternal cytotype on the number of pollen tubes observed (table 2). At each sampling point along the style, tetraploid pollen was more numerous than diploid pollen, regardless of the ploidy of the pollen recipient (figure 3). Moreover, the mean magnitude of difference between the number of tetraploid and diploid pollen tubes increased towards the base of the style, from 27% more than diploids at 6 mm to 58% at 8 mm, 86% at 10 mm and 174% at the style base. Pollen-tube number was higher in tetraploid mothers for both diploid and tetraploid pollen and for all four sampling points on the style; however, the difference was not statistically significant for any individual sampling distance (figure 3b). No differences in pollen tube number were observed among parents within maternal (except for 6 mm) or paternal cytotypes, nor was there a maternal $\times$ paternal interaction effect.

4. DISCUSSION

Post-mating, prezygotic interactions among male gametes, and between male gametes and the female reproductive tract, have been well documented as mechanisms regulating mating in populations of plants (Mulcahy & Mulcahy 1987; Snow & Spira 1991; Rigney *et al.* 1993; Smith-Huerta 1996). These processes have also been

recognized for over a century as potential mechanisms of reproductive isolation (Arnold 1997). However, their significance in plant and animal speciation has only recently been examined (Arnold 1997; Howard *et al.* 1998). This study represents, to our knowledge, the first investigation into gametic competition and reproductive isolation between a tetraploid plant and its diploid progenitor. We found that, in part, sexual isolation is indeed cryptic, operating after pollination but before fertilization within the pistil of the maternal plant. However, same-cytotype pollen was favoured in only one direction of the cross. Evidence for this unilateral siring advantage was reinforced by observations of pollen-tube growth in within- and between-cytotype pollinations. These results have important implications for polyploid dynamics and polyploid speciation.

To our knowledge, mixed pollinations have not been conducted for diploid progenitors and their polyploid descendants. Differential success of pollen has been studied for different subspecies (Nagy 1997) and congeneric species of plants (Arnold *et al.* 1993; Riesberg *et al.* 1995; Hauser *et al.* 1997), including two species of *Betula* that differ in ploidy (Williams *et al.* 1999). In each case, conspecific pollen always had a siring advantage, although the extent varied depending on the direction of the cross. In the only other study (to our knowledge) that is directly comparable with ours, Bogart (1980) examined the relative success of diploid and haploid sperm in frogs. He inseminated eggs from diploid *Hyla chrysoscelis* and its descendant tetraploid *Hyla versicolor* with a sperm mixture from the same species. He also found a unilateral advantage to one cytotype. In contrast to our results, it was haploid sperm that had complete siring success in both species.

Differential siring success may result from pollen–pistil incompatibilities, which block pollen, or from differential pollen-tube growth in the style. The fact that triploid offspring were produced in both directions of the diploid–tetraploid cross and that pollen tubes from diploids were observed in tetraploid styles indicates that there is no incompatibility blocking germination of haploid pollen on the stigma. Rather, the consistently high siring success of pollen from tetraploids appears to be facilitated by differential success of pollen tubes. After 6 h, pollen tubes from diploid and tetraploid pollen were observed in all style positions sampled. Moreover, the relative abundance of tetraploid pollen tubes increased toward the base of the style. Without sampling pollen tubes across a time interval, we cannot determine whether this pattern is due to differences in pollen-tube growth rates or to pollen-tube attrition. However, as pollen tubes from tetraploids dominate regardless of the cytotype of the pollen recipient, it is likely that the differential siring success is related to characteristics of the pollen rather than pollen–pistil interactions.

The unilateral success of pollen from tetraploids (i.e. diploid pollen) when in mixtures with pollen from diploids (haploid pollen) is unexpected given that gamete dysfunction, such as pollen sterility, is expected in pollen from autotetraploids, and single donor pollinations between cytotypes are frequently less fecund than crosses within cytotypes (Ramsey & Schemske 1998; Burton & Husband 2000). Two proximate reasons may explain the superiority

Table 2. Summary of ANOVA in pollen-tube number in crosses within and among diploid and tetraploid *Chamerion angustifolium*. Results are presented for four separate locations within the style. MS, mean squares.

source of variation	d.f.	MS	F
6 mm from stigma			
maternal cytotype	1	429.9	0.83
maternal plant (cytotype)	7	664.5	2.63*
paternal cytotype	1	1696.8	8.56**
paternal plant (cytotype)	7	167.2	0.66
maternal × paternal cytotype	1	178.5	0.71
8 mm from stigma			
maternal cytotype	1	1148.6	2.42
maternal plant (cytotype)	7	550.0	1.64
paternal cytotype	1	4620.6	22.63***
paternal plant (cytotype)	7	131.1	0.39
maternal × paternal cytotype	1	92.8	0.28
10 mm from stigma			
maternal cytotype	1	174.2	0.39
maternal plant (cytotype)	7	459.1	1.08
paternal cytotype	1	4904.7	24.85***
paternal plant (cytotype)	7	93.0	0.22
maternal × paternal cytotype	1	0.1	0.0001
base of style			
maternal cytotype	1	393.5	1.18
maternal plant (cytotype)	7	376.6	1.49
paternal cytotype	1	5636.8	34.59***
paternal plant (cytotype)	7	112.0	0.44
maternal × paternal cytotype	1	175.1	0.69

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

of diploid pollen. First, diploid pollen grains may grow faster because they are better able to shelter deleterious alleles (Otto & Whitton 2000). Because each pollen grain carries two copies of every gene, the negative effects of recessive or partially recessive mutations will be masked in pollen from tetraploids, whereas these same mutations would be immediately expressed in haploid pollen. These sheltering effects are expected to be transient, as deleterious mutations will eventually accumulate in tetraploids, but the effect on the gametophyte should persist for many generations (Otto & Whitton 2000). Second, pollen from tetraploid plants may sire more seeds than pollen from diploids because, on average, they are larger in size. Pollen size may be related to siring ability in many plant species (Manicacci & Barrett 1995). Surveys of pollen grain size (or volume) in many taxonomic groups have revealed positive correlations with pistil length (Williams & Rouse 1990; Sarkissian & Harder 2001) and pollen-tube growth rates (Williams & Rouse 1990). More direct investigations of siring ability have also shown positive correlations with pollen size (Manicacci & Barrett (1995), but see Cruzan (1990)). Although the basis for this association is not well understood, many researchers have suggested that pollen size reflects the quantity of resources available for pollen-tube growth. Further studies are required to distinguish these possible mechanisms. Given the association between large pollen and polyploidy in plants, pollen precedence may well have a general role in reproductive isolation and evolution of polyploidy in many plants.

Polyploidy may be a significant mechanism of speci-

ation. Implicit in this perspective is the assumption that reproductive isolation arises in association with chromosome doubling. However, few studies have examined the extent of reproductive isolation between related cytotypes or identified the kinds of barriers to reproduction operating between two taxa (Husband & Schemske 2000). This is especially important in autopolyploids, which often resemble their diploid progenitors morphologically and thus are frequently grouped within the same taxonomic species. Our results confirm that gametic interactions form an important reproductive barrier between the diploid and autotetraploid *C. angustifolium*. However, due to unilateral pollen precedence, tetraploids experience greater isolation than diploids. Based on pollen competition alone, and assuming that pollen from both cytotypes reaches all plants in a population, tetraploids should mate assortatively for 79% of the time, while diploids will mate assortatively for 39% of the time. Other prezygotic barriers exist that further reduce the likelihood of between-cytotype mating in sympatric populations. In a previous study of a mixed cytotype population of *C. angustifolium*, we found that diploids and tetraploids mate assortatively for 13% and 6% of the time, respectively, owing to flowering time differences (Husband 2000; Husband & Schemske 2000). Of those overlapping in flowering, 43% and 80% of diploids and tetraploids, respectively, mate assortatively due to pollinator fidelity (Husband & Schemske 2000). Collectively, these reproductive barriers reduce the likelihood of between-cytotype mating to 4% in tetraploids and 30% in diploids. This indicates that, based on prezygotic bar-

Figure 3. Mean ($\pm$ s.e.) number of pollen tubes for (a) diploid and (b) tetraploid maternal parent plants of *Chamerion angustifolium* pollinated with diploid (open bars) or tetraploid (grey bars) pollen. Pollen-tube counts at 6 mm, 8 mm and 10 mm from the stigma and at the style base are presented.

riers alone, tetraploids are nearly completely isolated from diploids, but that some gene movement can occur from tetraploids to diploids. Gene flow is likely to be reduced further by the presence of strong postzygotic barriers, which reduce the viability and fertility of triploids to 9% of that of diploids (Burton & Husband 2000).

Asymmetrical sexual isolation may provide clues about the origins of species. It has been proposed that mating asymmetries are ephemeral and will decay as populations diverge (Arnold *et al.* 1996); however, these statements pertain to premating barriers. Asymmetries in gametic competition may also arise early in the divergence of two populations, the direction of the asymmetry perhaps being an epiphenomenon of chromosome doubling itself. As mentioned, doubling may result in increased pollen size and hence increased pollen-tube growth rates or increased sheltering of deleterious mutations. This effect may weaken with time or be masked by the development of stronger species recognition and rejection responses typical of fully diverged species. Based on this argument, the unilateral siring patterns observed indicate that polyploid *C. angustifolium* may have diverged only recently from the diploid ancestor. Alternatively, the mating asymmetry may be a product of divergent selection on pollen–pistil interactions. For example, pollen produced by tetraploids may experience a very different selective environment than diploid pollen owing to the fact that tetraploid flowers are

generally larger, with longer styles (Husband & Schemske 2000). Other studies have found a strong correlation between pollen size and style length (Blakeslee 1945; Williams & Rouse 1990; Torres 2000; Sarkissian & Harder 2001). Such differences in pollen performance may persist or even increase with increased divergence between the cytotypes. Comparative studies of polyploids that have diverged to different degrees from their diploid progenitor will be necessary to determine the relative importance of these mechanisms.

By creating an asymmetrical pattern of isolation and fertilization, unilateral pollen precedence has important implications for the evolutionary dynamics of polyploids in sympatry with diploids. Theoretical studies show that newly formed polyploids will experience a strong fitness disadvantage due to the swamping effects of diploid pollen and the fact that most ovules will be usurped by triploid fertilizations, which are generally less viable (Levin 1975; Felber 1991; Husband 2000). However, through the effects of pollen precedence, polyploids may compensate in two distinct ways: first by ensuring that polyploid mothers produce polyploid offspring, and second by increasing the number of hybrid matings in diploids. In a previous study involving experimental populations of *C. angustifolium* with different proportions of diploid and tetraploid plants, we found frequency-dependent seed production in diploids but not in tetraploids (Husband 2000). This pattern may be explained in part by the unilateral siring success of tetraploids and the fact that tetraploids will have a much larger impact on seed production in diploids than diploids will have on tetraploids (Nagy 1997). This may diminish the relative selective advantage of diploids and increase the opportunities for the spread and persistence of neotetraploids in diploid populations.

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REFERENCES

- Arnold, M. L. 1997 *Natural hybridization and evolution*. Oxford University Press.
- Arnold, M. L., Hamrick, J. L. & Bennett, B. D. 1993 Interspecific pollen competition and reproductive isolation in *Iris*. *J. Hered.* **84**, 13–16.
- Arnold, S. J., Verrell, P. A. & Tilley, S. G. 1996 The evolution of asymmetry in sexual isolation: a model and a test case. *Evolution* **50**, 1024–1033.
- Blakeslee, A. F. 1945 Removing some of the barriers to crossability in plants. *Proc. Am. Phil. Soc.* **89**, 561–574.
- Bogart, J. P. 1980 Evolutionary implications of polyploidy in amphibians and reptiles. In *Polyploidy, biological relevance* (ed. W. H. Lewis), pp. 341–378. New York: Plenum.
- Burton, T. L. & Husband, B. C. 1999 Population cytotype structure in the polyploid *Galax urceolata* (Diapensiaceae). *Heredity* **82**, 381–390.
- Burton, T. L. & Husband, B. C. 2000 Fitness differences among diploids, tetraploids, and their triploid progeny in *Chamerion angustifolium*: mechanisms of inviability and implications for polyploidy evolution. *Evolution* **54**, 1182–1191.
- Burton, T. L. & Husband, B. C. 2001 Fecundity and offspring ploidy in matings among diploid, triploid and tetraploid

- Chamerion angustifolium* (Onagraceae): consequences for tetraploid establishment. *Heredity* **87**, 573–582.
- Cruzan, M. B. 1990 Variation in pollen size, fertilization ability, and post-fertilization siring ability in *Erythronium grandiflorum*. *Evolution* **44**, 843–856.
- Felber, F. 1991 Establishment of a tetraploid cytotype in a diploid population: effect of relative fitness of the cytotypes. *J. Evol. Biol.* **4**, 195–207.
- Flint, E. 1980 Ecology and distribution of diploid and tetraploid *Epilobium angustifolium* (fireweed) in the Beartooth Mountains of Wyoming and Montana. PhD dissertation, Duke University, Durham, NC, USA.
- Hauser, T. P., Jorgensen, R. B. & Ostergard, H. 1997 Preferential exclusion of hybrids in mixed pollinations between oilseed rape (*Brassica napus*) and weedy *B. campestris* (Brassicaceae). *Am. J. Bot.* **84**, 756–762.
- Howard, D. J. & Gregory, P. G. 1993 Post-insemination signaling systems and reinforcement. *Phil. Trans. R. Soc. Lond. B* **340**, 231–236.
- Howard, D. J., Reece, M., Gregory, P. G., Chu, J. & Cain, M. L. 1998 The evolution of barriers to fertilization between closely related organisms. In *Endless forms: species and speciation* (ed. D. J. Howard & S. H. Berlocher), pp. 279–288. Oxford University Press.
- Husband, B. C. 2000 Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. *Proc. R. Soc. Lond. B* **267**, 217–223. (DOI 10.1098/rspb.2000.0990.)
- Husband, B. C. & Schemske, D. W. 1998 Cytotype distribution at a diploid–tetraploid contact zone in *Chamerion (Epilobium) angustifolium* (Onagraceae). *Am. J. Bot.* **85**, 1688–1694.
- Husband, B. C. & Schemske, D. W. 2000 Ecological mechanisms of reproductive isolation between diploid and tetraploid *Chamerion angustifolium*. *J. Ecol.* **88**, 689–701.
- Levin, D. A. 1975 Minority cytotype exclusion in local plant populations. *Taxon* **24**, 35–43.
- Manicacci, D. & Barrett, S. C. H. 1995 Stamen elongation, pollen size, and siring ability in tristylous *Eichhornia paniculata* (Pontederiaceae). *Am. J. Bot.* **138**, 1381–1389.
- Martin, F. M. 1959 Staining and observing pollen tubes in the style by means of fluorescence. *Stain Technol.* **34**, 436–437.
- Mosquin, T. 1966 A new taxonomy for *Epilobium angustifolium* L. (Onagraceae). *Brittonia* **18**, 176–188.
- Mulcahy, D. L. & Mulcahy, G. B. 1987 The effects of pollen competition. *Am. Sci.* **75**, 44–49.
- Nagy, E. S. 1997 Frequency-dependent seed production and hybridization rates: implications for gene flow between locally adapted plant populations. *Evolution* **51**, 703–714.
- Otto, S. P. & Whitton, J. 2000 Polyploid incidence and evolution. *A. Rev. Gen.* **34**, 401–437.
- Ramsey, J. & Schemske, D. W. 1998 Pathways, mechanisms, and rates of polyploid formation in flowering plants. *A. Rev. Ecol. Syst.* **29**, 467–501.
- Riesberg, L. H., Desrochers, A. M. & Youn, S. J. 1995 Interspecific pollen competition as a reproductive barrier between sympatric species of *Helianthus* (Asteraceae). *Am. J. Bot.* **82**, 515–519.
- Rigney, L. P., Thomson, J. D., Cruzan, M. B. & Brunet, J. 1993 Differential success of pollen donors in a self-compatible lily. *Evolution* **47**, 915–924.
- Sarkissian, T. S. & Harder, L. D. 2001 Direct and indirect responses to selection on pollen size in *Brassica rapa* L. *J. Evol. Biol.* **14**, 456–468.
- Schluter, D. 1994 Experimental evidence that competition promotes divergence in adaptive radiation. *Science* **266**, 798–801.
- Smith, E. B. 1968 Pollen competition and relatedness in *Haplopappus* section *Isopappus*. *Bot. Gaz.* **129**, 371–373.
- Smith-Huerta, N. L. 1996 Pollen germination and tube growth in selfing and outcrossing populations of *Clarkia tembloriensis*. *Int. J. Plant Sci.* **157**, 228–233.
- Snow, A. A. & Spira, T. P. 1991 Differential pollen-tube growth rates and non-random fertilization in *Hibiscus moscheutos* (Malvaceae). *Am. J. Bot.* **78**, 1419–1426.
- Torres, C. 2000 Pollen size evolution: correlation between pollen volume and pistil length in Asteraceae. *Sexual Plant Reprod.* **12**, 365–370.
- Williams, E. G. & Rouse, J. L. 1990 Relationships of pollen size, pistil length and pollen tube growth rates in *Rhododendron* and their influence on hybridization. *Sexual Plant Reprod.* **3**, 7–17.
- Williams Jr, J. H., Friedman, W. E. & Arnold, M. L. 1999 Developmental selection within the angiosperm style: using gamete DNA to visualize interspecific pollen competition. *Proc. Natl Acad. Sci. USA* **96**, 9201–9206.

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