Polyploidy and ecological adaptation in wild yarrow

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Chromosome evolution in flowering plants is often punctuated by polyploidy, genome duplication events that fundamentally alter DNA content, chromosome number, and gene dosage. Polyploidy confers postzygotic reproductive isolation and is thought to drive ecological divergence and range expansion. The adaptive value of polyploidy, however, remains uncertain; ecologists have traditionally relied on observational methods that cannot distinguish effects of polyploidy per se from genic differences that accumulate after genome duplication. Here I use an experimental approach to test how polyploidy mediates ecological divergence in *Achillea borealis* (Asteraceae), a widespread tetraploid plant with localized hexaploid populations. In coastal California, tetraploids and hexaploids occupy mesic grassland and xeric dune habitats, respectively. Using field transplant experiments with wild-collected plants, I show that hexaploids have a fivefold fitness advantage over tetraploids in dune habitats. Parallel experiments with neohexaploids—first-generation mutants screened from a tetraploid genetic background—reveal that a 70% fitness advantage is achieved via genome duplication per se. These results suggest that genome duplication transforms features of *A. borealis* in a manner that confers adaptation to a novel environment.

biogeography | ecological speciation | range boundary

Polyploidy is a conspicuous and recurrent form of genetic variation in flowering plants (1–3). Since its discovery in the early 20th century, polyploidy has been postulated to mediate ecological differentiation, for three reasons. First, experimental polyploids in crop species exhibit altered morphological and growth characteristics (4–7). Second, in nature, closely related diploid and polyploid species have distinct geographic ranges (8, 9). Third, the occurrence of polyploidy varies across geographic regions, with highest incidences reported in alpine and arctic floras (10). Many evolutionists believe that polyploidy imbues plants with novel features that allow them to invade new environments or expand their geographic range (11–14). This hypothesis, however, has not been tested rigorously. Ecological studies have traditionally focused on simple comparisons of diploids and naturally occurring polyploids that diverged in the distant past (15–20). This approach confounds phenotypic differences caused by polyploidy with those caused by genic changes arising since the time of polyploid formation (21–23).

Here I use an experimental approach to untangle the contributions of polyploidy per se to ecological divergence. Although sometimes regarded as a macroevolutionary event, genome duplication is an ongoing process in many plant populations (6, 24). First-generation polyploid mutants ("neopolyploids") form at rates equal to or exceeding the per locus mutation rate, and can thus be identified by *en masse* screening using flow cytometry (25, 26). Because neopolyploids share a common genetic background with their diploid progenitors, the comparison of these cytotypes provides a direct measure of the phenotypic effects of genome duplication as influenced by cell size, cell geometry, and gene dosage (6, 7, 27). In contrast, comparison of neopolyploids with established polyploid populations reveals the contributions of allele substitutions, gene silencing, and other genic changes that evolved subsequent to genome duplication.

I performed field experiments with neopolyploids and wild-collected polyploids of wild yarrow. *Achillea borealis* (Asteraceae) is an autopolyploid complex of tetraploid ($2n = 4x = 36$) and hexaploid ($2n = 6x = 54$) populations that is endemic to North America (28–30). These cytotypes are reproductively isolated by partial inviability and sterility of pentaploid ($2n = 5x = 45$) hybrids (31, 32). Hexaploids reside in Mediterranean environments (winter wet, summer dry) on the western coast of North America, including sand dunes and oak woodlands, but tetraploids inhabit more mesic environments, like coastal grasslands, coniferous forest, and alpine meadows (28, 33). The two cytotypes occur in close proximity in parts of California, Oregon, and Washington state (Fig. 1*A*) (30, 32, 34).

My studies were conducted on a 200-km stretch of California's north coast and had three primary components. First, to provide an ecological context for field experiments, I characterized the spatial distributions and habitat associations of tetraploid and hexaploid *A. borealis*. Second, I compared survivorship of wild-collected tetraploids and hexaploids that were experimentally transplanted to a dune environment normally inhabited only by hexaploids. Finally, I transplanted related tetraploid and neohexaploid plants to the dune environment previously used to compare wild-collected tetraploids and hexaploids. This research tests the contributions of polyploidy per se to ecological divergence, although it does not evaluate the performance of neohexaploids in the grassland environments from which they originate.

Results

Spatial Distribution of Cytotypes. No sympatric occurrences were found among 540 cytotyped plants at 18 sites: populations were comprised of either tetraploids or hexaploids. Tetraploid and hexaploid populations were found primarily in the northern and southern portions of the study region, respectively (Fig. 1*B*). Interdigitation was observed, however, with three hexaploid sites extending north of the most southerly tetraploid population, and two tetraploid sites occurring south of the most northerly hexaploid population (Fig. 1*B*).

Soil, Vegetation, and Habitat Associations. Soils in tetraploid sites had more organic matter (12.7 vs. 1.1%) and gravel (54.1 vs. 2.8%) but less sand (71.8 vs. 96.0%) than soils in hexaploid sites ($n = 18$ sites, Mann Whitney U tests, $P < 0.001$; see *Methods*). A total of 172 vascular plant taxa were surveyed within the 18 study populations, with tetraploid sites harboring more species than hexaploid sites (mean 62.1 vs. 49.1; $n = 18$ sites, Mann-Whitney U test, $Z = -3.007$, $P = 0.001$). In vegetation ordinations, tetraploid and hexaploid *A. borealis* were allied with different plant taxa. Hexaploid *A. borealis* was associated with sand dune specialists, including *Tanacetum camphoratum* (dune tansy; eight of nine hexaploid sites), *Camissonia cheiranthifolia* (beach evening primrose; eight of nine hexaploid sites), *Abronia latifolia* (yellow sand verbena; seven of nine sites), and *Ambrosia chamissonis* (beach bur; seven of nine hexaploid sites) (35, 36). Tetraploid *A. borealis* was associated with grassland and forest edge species,

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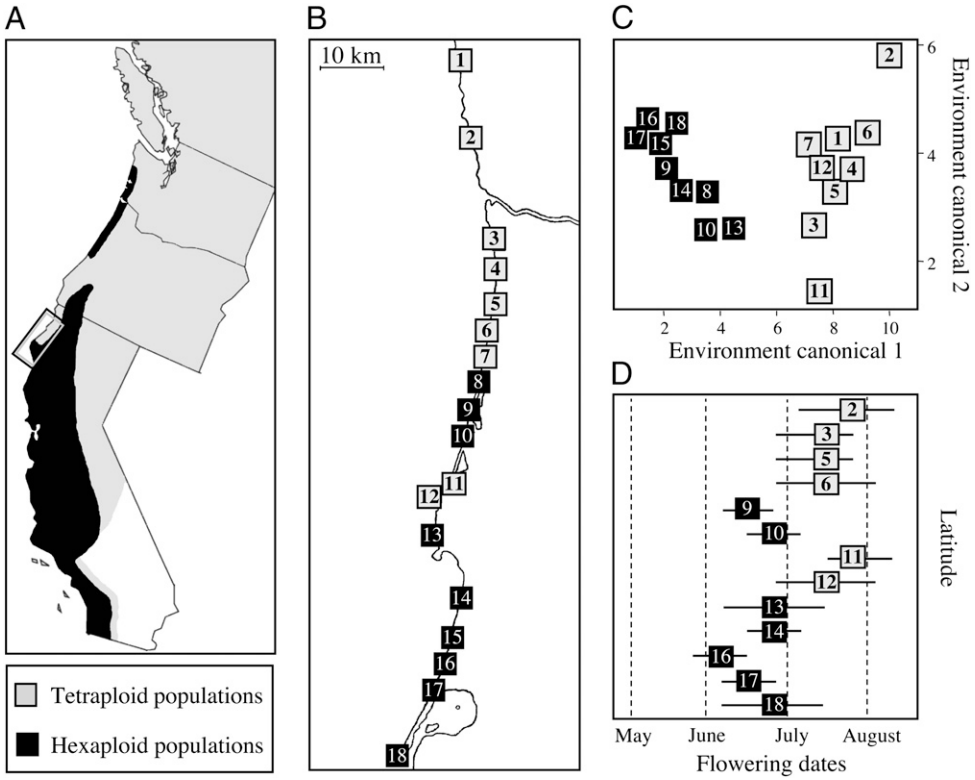

Fig. 1. Cytotype distribution and habitat associations. Numbers correspond to population designations in the text. (*A*) Geographic distribution of *A. borealis* cytotypes in western North America. (*B*) Distribution of tetraploid and hexaploid populations in study region, 200 km of California's northern coast. (*C*) Discriminant function analysis based on soil texture, organic content, and vegetation; the model correctly classified ploidy level of all sites. Tetraploid and hexaploid sites represent grasslands and dune habitats, respectively. (*D*) Flowering phenology of study sites. Icons indicate times of peak reproduction and lines show dates when >50% of plants were flowering. Tetraploid populations flowered 1 mo after hexaploid populations.

including *Angelica hendersonii* (sea watch; nine of nine tetraploid sites), *Equisetum telmateia* (giant horsetail; nine of nine tetraploid sites), *Lupinus rivularis* (riverbank lupine; nine of nine sites), and *Heracleum lanatum* (cow parsnip; eight of nine tetraploid sites).

Discriminant function analysis indicated that tetraploid and hexaploid sites were distinguished by soil factors and vegetation composition (Wilk's Λ=0.093, $F_{3,14}$ = 45.269, $P < 0.001$) (Fig. 1*C*). Vegetation composition and soil texture were correlated with the discriminant function's primary axis, which explained 100% of the variance between cytotype habitats. Tetraploid sites were densely vegetated coastal grasslands, associated with rocky headlands and sea cliffs; hexaploid sites were sandy, sparsely vegetated beaches and dune systems (Fig. 2 *A* and *B*). Flowering phenology differed between the ploidy levels, with tetraploid populations reaching peak flowering 29 d after hexaploid populations (n = 13 sites, Mann Whitney U test, Z = –3.095, P =

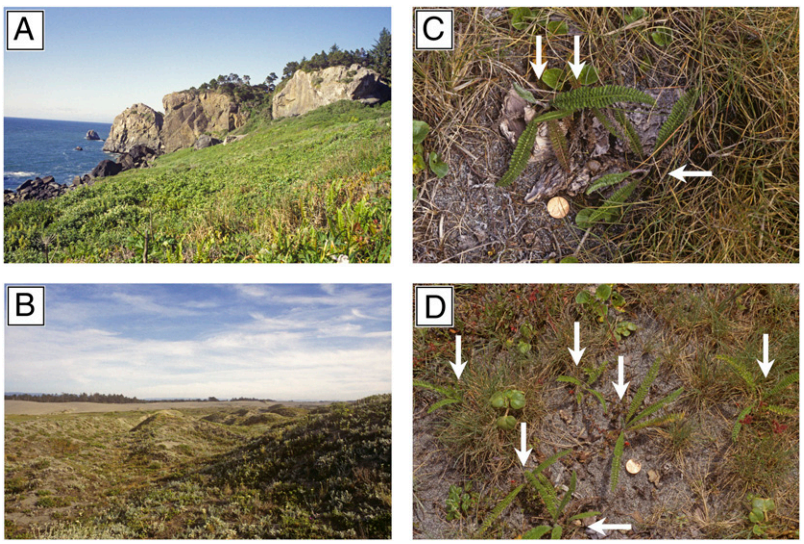

Fig. 2. Photographs of study sites and experimental transplants. (*A*) Coastal headland and associated grassland inhabited by tetraploid *A. borealis* (Patrick's Point, CA; study site 12). (*B*) Dune system inhabited by hexaploid *A. borealis* (Lanphere Dunes, CA; study site 16). (*C*) Neohexaploid transplant to dune environment [three stems (arrows); coin for scale]. (*D*) Established hexaploid transplant to dune environment [six stems (arrows); coin for scale].

0.001) (Fig. 1*D*). There was no apparent relationship between latitude and flowering phenology in the study area (Fig. 1).

Transplantation Experiments (Wild-Collected Plants). To test if hexaploid *A. borealis* is intrinsically adapted to dune environments, I performed a 3-year field transplant experiment using seedlings from multiple populations of both cytotypes (Figs. 2 and 3 *A* and *B*) (see *Methods*). On average, hexaploids had fivefold higher survival than tetraploids (mean survivorship 26.4 vs. 4.4%; n = 2,150 transplants, logistic regression, likelihood-ratio test, $\chi^2 = 33.155$, $P < 0.001$) (Fig. 3*A* and Table 1). Hexaploids native to the transplant site exhibited the highest survivorship (35.9%) but tetraploids originating furthest from the transplant site exhibited the lowest survivorship (2.6%). Mortality of dune transplants coincided with the summer months, when many plants succumbed to water stress. For example, survivorship through the rainy season (November to April) and the dry season (May to October) averaged 95.0 and 44.7% per year, respectively. On average, hexaploid transplants flowered 3 wk earlier in the season than tetraploid transplants, which were rarely reproductive (n = 68 flowering stems, Mann Whitney U test, $Z = -2.327$, $P = 0.001$) (Fig. 3*B*). Transplant flowering phenology was similar to that observed in natural tetraploid and hexaploid populations (Figs. 1*D* and 3 *B* and *D*).

Transplantation Experiments (Neopolyploids). To determine the contribution of polyploidy per se to adaptation, I performed transplant experiments in the aforementioned dune environment with neohexaploid plants (Figs. 2 and 3 *C* and *D*) (see *Methods*). Neohexaploids experienced a 70% survival advantage over their tetraploid siblings, accounting for almost one-third of the fitness difference between naturally occurring tetraploids and hexaploids that were planted as experimental controls (mean survivorship 20.4% vs. 34.7% vs. 70.4%; n = 1,023 transplants; logistic regression, likelihood-ratio test, $\chi^2 = 8.605$, $P = 0.003$) (Fig. 3*C* and Table 1). On average, neohexaploid transplants flowered 9 d earlier in the season than tetraploids and 12 d later than hexaploids (n = 95 reproductive stems; Mann Whitney U tests, $P < 0.01$) (Fig. 3*D*).

Discussion

Polyploidy has occurred repeatedly during the evolutionary history of vascular plants (1–3). Among angiosperms, whole-genome duplication is thought to have happened twice before the divergence of Eudicots and Monocots in the late Jurassic, and thereafter one or more times independently within many flowering plant lineages between the late Cretaceous and mid-Tertiary (37, 38). Polyploidy is regarded as a primary mechanism of speciation among modern-day angiosperms, with as many as 15% of recent speciation events associated with polyploidization (3, 39–41). Cultivated wheat, maize, cotton, potato, tobacco, cabbage, and strawberries are polyploids, and many diploid crops have polyploid relatives of agronomic importance (1, 42). Because its incidence varies by latitude and elevation, polyploidy is thought to imbue plants with novel features that allow them to occupy new environments, expand their geographic range, or achieve new species interactions (5, 11, 12, 43). The adaptive significance of polyploidy remains untested, however, because ecological research has historically involved comparisons of diploids and polyploids that diverged tens or hundreds of thousands of years in the past (9). Such studies cannot distinguish the effects of polyploidy per se (cell size, DNA content, gene dosage) from genic evolution

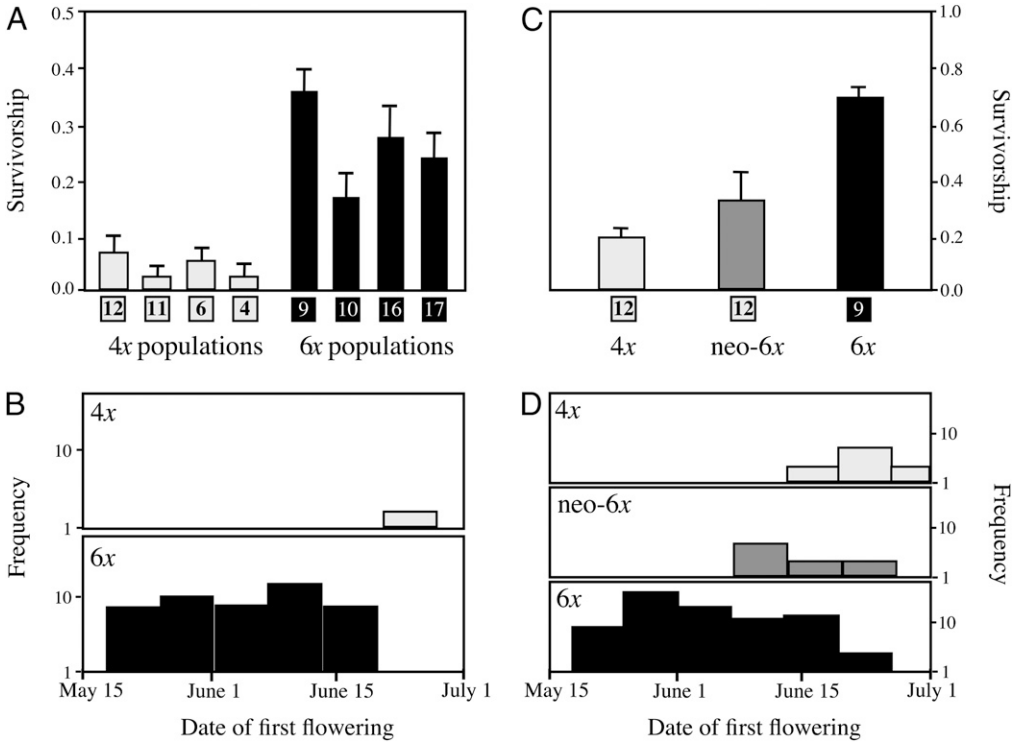

Fig. 3. Fitness and phenology of experimental transplants to dune (hexaploid) environment. Numbers correspond to population designations in the text. (*A*) Survivorship of seedlings from natural tetraploid and hexaploid populations (mean + 2 SEM). On average, hexaploids had a fivefold fitness advantage (Table 1). (*B*) Transplant flowering phenology. Hexaploids flowered earlier than tetraploids and more frequently. (*C*) Survivorship of neohexaploids and experimental controls planted as vegetative cuttings (mean + 2 SEM). Neohexaploids had 70% greater survivorship than tetraploids, accounting for almost one-third of the fitness difference between tetraploids and established hexaploids. (*D*) Flowering of neohexaploids and experimental controls. Neohexaploid flowering phenology was intermediate to that of tetraploids and established hexaploids.

Table 1. Statistical analyses of survivorship in dune transplant experiments

Comparison	Result	Effect test	χ^2 (df)	*P*
Tetraploids vs. hexaploids	Likelihood ratio test, $\chi^2 = 446.197$, df = 16, $P < 0.0001$	Ploidy	33.155 (1)	<0.0001
		Population [Ploidy]	38.360 (7)	<0.0001
		Plot	193.558 (8)	<0.0001
Tetraploids vs. neohexaploids	Likelihood ratio test, $\chi^2 = 105.115$, df = 14, $P < 0.0001$	Ploidy	8.605 (1)	0.0034
		Maternal parent	21.060 (4)	0.0003
		Plot	79.284 (9)	<0.0001

that occurs in all lineages, irrespective of ploidy (allele substitution, gene silencing, gene duplication) (6, 7). Consequently, studies of natural-occurring polyploids tend to overestimate the phenotypic and ecological consequences of genome duplication (22, 27, 44).

Achillea borealis belongs to the *Achillea millefolium* aggregate, a circumpolar group of 15 species (30, 45). Phylogenetic analyses indicate that *Achillea* colonized the New World in the recent geologic past (Pleistocene) via the Alaskan land bridge (30). The tetraploid colonists spread rapidly throughout North America, evolving into an assemblage of ecological races adapted to grasslands, salt marshes, open coniferous forests, alpine meadows, and other habitats (28–30, 46). Despite this broad ecological distribution, only hexaploids occur in Mediterranean habitats of the Pacific Coast (coastal dunes, oak woodlands, bottomlands of the Central Valley) (28, 32, 34, 46). Tetraploids and hexaploids appear to have strong ecological boundaries despite cooccurrence in zones of sympatry, with cytotype populations segregating between xeric and mesic environments (Fig. 1).

In the experiments reported here, neopolyploids recapitulated ecological adaptations of naturally occurring polyploids, suggesting that genome duplication facilitated the colonization of Mediterranean environments. This finding corroborates the long-standing belief that polyploidy per se can mediate ecological divergence in plants (4, 11). Nonetheless, performance differences between tetraploids and naturally occurring hexaploids exceeded those between tetraploids and neohexaploids. Phenotypic characteristics of hexaploids thus appear to have been shaped by genic evolution after the time polyploid formation, in the context of reinforcement (selection to avoid production of sterile intercytotype hybrids), character displacement (selection to minimize competition with progenitors), or fine-scale adaptation to the environments in which they now occur (6, 7, 17, 21).

The physiological mechanisms underlying the performance of established hexaploids probably involve water relations, drought resistance, and seasonal dormancy. Because DNA content affects the size and geometry of guard cells and xylem elements, polyploidy is hypothesized to alter transpiration rates and water-use efficiency in plants (44, 47). It is also possible that hexaploids have an intrinsic fitness advantage over tetraploids because the addition of chromosome complements masks the effects of recessive deleterious alleles (2, 6, 7). In this scenario, polyploidy may facilitate colonization of novel environments by increasing mean population fitness rather than conferring specific "preadaptive" traits. Common garden studies are now underway to compare physiological and life-history features of neohexaploids and established hexaploids in *A. borealis*.

Despite the viability of neohexaploid plants—and the spatial proximity of tetraploid and established hexaploid populations—hexaploids have not been recovered from tetraploid sites except when screening spontaneous neopolyploid mutants (26, 30). The establishment of hexaploids is probably prevented by minority cytotype exclusion, positive frequency-dependent selection caused by reproductive incompatibility between plants of differing ploidy level (19). Theoretical models suggest that, in the absence of self-fertilization, polyploid establishment occurs only when rates of formation are unusually high (>5%) and polyploids have a marked fitness advantage (48, 49). Although the experiments reported here indicate that neohexaploids outperform their tetraploid relatives in a dune habitat, it is unclear if this advantage extends to grassland habitats where neohexaploids form. Thus, the absence of hexaploids in tetraploid populations may reflect minority cytotype exclusion, the intrinsic physiological and life-history characteristics of hexaploid plants, or both factors.

Ecological investigation of neopolyploids is in its infancy, and to my knowledge, there are no comparable field experiments from other plant taxa. Greenhouse and garden studies report qualitatively similar findings as those described here (22, 23, 44). Colchicine-induced neotetraploids of fireweed (*Chamerion angustifolium*, Onagraceae), for example, have hydraulic conductivities (K_H) and time-to-wilt values intermediate to diploids and established tetraploids. On the other hand, neotetraploids have significantly larger leaves and stomatal length than either diploids or established tetraploids (44). Genome duplication transforms phenotypic features of *C. angustifolium* yet, as reported here for *A. borealis*, neopolyploids exhibit numerous phenotypic differences to established polyploids.

Experimental studies of neopolyploids provide critical insights into the relationship between polyploidy and adaptation. The approach is not perfect, however, and several caveats are acknowledged. First, polyploidy may originate in a genetic background that responded to genome duplication somewhat differently than current-day populations that are selected for study. Second, competition with progenitor cytotypes may "sieve" neopolyploids such that only the most divergent individuals contribute to demographic establishment. Thus, the phenotypic divergence of randomly-selected neohexaploids could underestimate the divergence of ecologically successful neohexaploids. Finally, genetic features of neopolyploids may contribute to their evolvability over time. For example, polyploid populations may exhibit a more rapid response to natural selection than diploid populations because of multisomic inheritance or epigenetic processes (2, 6, 7). Increased sampling of neopolyploid genotypes—within and between populations, and across multiple generations—are needed to address these issues.

Methods

Geographic Variation in Ploidy. Eighteen study sites were established in natural habitats along a 200-km stretch of the California coastline that previous sampling suggested would contain tetraploids and hexaploids (30, 32, 34). Ploidy determinations were made by flow cytometry analysis of greenhouse-grown seedlings, using previously described methods (26). One seedling was cytotyped from 30 randomly selected maternal parents per study population.

I collected soil samples using a corer ($5 \times 5 \times 20$ cm) at 30 positions per study site. Samples were air-dried and pooled into single collections for analysis. Gravel and rock content was measured using #10 sieves and summarized as the proportion of total sample weight comprised of particles >2 mm in diameter. Particle size distribution of sieved soil was measured using mechanical (sedimentation) analysis, with sample composition reported as percent sand (2–0.05 mm), silt (0.05–0.002 mm), and clay (<0.002 mm). Loss-at-ignition was determined as weight loss following 6 h heating at 500 °C (50). Because multiple trait comparisons were made for soils, I used a Bonferroni correction to identify an appropriate significance level ($P = \alpha/n = 0.0125$).

Presence-absence inventories of vascular plants were made in 50 × 150-m plots that circumscribed the study populations of *A. borealis*. Inventories were conducted biweekly from April to August. Plant identifications were based on *The Jepson Manual of the Higher Plants of California* (35). Site and species ordinations were generated using detrended correspondence analysis (51) with rescaled axes, calculated with the PC-Ord software package (v. 5.0; MjM software). I used discriminant functional analysis to test whether tetraploid and hexaploid sites were distinguished statistically by soil features and vegetation. The number of taxa in the vegetation matrix exceeded the number of study sites, so I included the primary axis score of the vegetation ordination in the discriminant model (52). Loss-at-ignition and one measure of soil texture (percent sand) were also included in the model, which was performed using the JMP statistical package (v. 9.0; SAS Institute).

Flowering phenology of 13 populations was monitored in randomly-positioned quadrats at 10-d intervals (April to August). Total quadrat area per site was ~1,500 m^2 and contained 1,000 to 3,500 flowering ramets. I summarized flowering phenology by dividing the number of plants flowering at each census date by the total number of flowering stems present in quadrats at each study site.

Plant Materials. Experimental studies of tetraploids and hexaploids included eight populations, including populations 4, 6, 9, 10, 11, 12, 16, and 17, that spanned the latitudinal range of the study region (Fig. 1). I sampled 10 maternal families from each population; transplant studies of this material were established using seedlings. Studies of neopolyploids included plants from two populations (populations 9 and 12). Tetraploids (41 genotypes) and neohexaploids (six genotypes) were previously identified (26) among progeny of five tetraploid maternal parents from population 12; hexaploids from six maternal families (21 genotypes) in nearby population 9 were also included to represent characteristics of naturally occurring hexaploids. Transplant studies of neohexaploids and experimental controls were established using vegetative clones. All transplant experiments were conducted at site 9, a representative dune (hexaploid) habitat located in the middle of the study area (Fig. 1).

All transplant materials were grown in the greenhouse. Seeds were sown into peat-perlite soil mix in plug trays and misted daily until germination. I irrigated seedlings daily in complete nutrient solution (100 ppm nitrogen). Supplemental lighting was provided by high-pressure sodium lights with an average intensity of 60 W per square meter. Transplant experiments with tetraploids and hexaploids were performed with seedlings (6-wk old) that had four or five true leaves and an established root system. Because transplant experiments with neohexaploids and controls used vegetative clones, this material was moved to larger pots (12 × 12 × 12 cm) and serially propagated. Plants were then trimmed to synchronously induce growth of lateral meristems. I matched harvested shoots for size (three or four leaves) and planted them in rose pots (5 × 5 × 5 cm). Rooting of cuttings was highly successful (>80%) and did not differ among cytotypes.

Transplant Experiments. I initiated both field transplantation experiments during the wet season (early February). Transplants were randomized and directly planted into native soil on a 50-cm grid system. To reduce early belowground competition, I mechanically stirred soil in a 10-cm radius around each transplant position before planting. Transplants were planted 2 cm below grade and surrounded with bark chips (Douglas fir) for mulch. I performed weekly irrigations (no nutrient additions) for 2 mo to assist establishment. Early mortality was low in both experiments (<10%, February to May).

For experiments with tetraploids and hexaploids, nine plots of ~240 plants (three seedlings in 10 maternal families from eight populations) were established (2,150 total seedlings). For experiments with neohexaploids and controls, 10 plots of ~100 plants were established (409 tetraploid, 322 neohexaploid, and 292 hexaploid cuttings). *A. borealis* is a long-lived perennial, so both experiments were maintained for a 3-y period. I monitored survival and clonal growth of transplants by seasonal examination of belowground rhizome connections, which were marked with plastic toothpicks to facilitate future identification. Surrounding vegetation competed with transplants but was pruned each spring to facilitate stem identification. I covered inflorescences of reproductive stems with bridal veil to prevent pollen flow with native populations at the transplant site, as per requirements of permits issued by California State Parks. Wild yarrow is self-incompatible, and the covering of inflorescences prevented the measurement of transplant fertility and fecundity.

Transplants exhibited high mortality and slow biomass accrual, so fitness was summarized as survivorship to the conclusion of experiments (tetraploids and hexaploids: 329 survivors of 2,150 planted seedlings; neohexaploids and controls: 409 survivors of 1,023 planted cuttings). I monitored flowering phenology of the few surviving plants in the third year of the experiment from April to July, corresponding to the normal flowering period in dune habitats (Fig. 1). Date of first flowering was determined for reproductive stems by censuses conducted on 3-d intervals (tetraploids and hexaploids: 68 flowering stems on 40 reproductive transplants; neohexaploids and controls: 95 flowering stems on 72 reproductive transplants). Statistical analyses of survivorship were performed with JMP and used logistic regression. For comparison of tetraploids and hexaploids, I included ploidy, population (nested under ploidy), and plot as factors. For comparison of tetraploids and neohexaploids, I included ploidy, maternal family, and plot as factors.

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1. Grant V (1981) *Plant Speciation.* (Columbia University Press, New York).
2. Otto SP, Whitton J (2000) Polyploid incidence and evolution. *Annu Rev Genet* 34: 401–437.
3. Wood TE, et al. (2009) The frequency of polyploid speciation in vascular plants. *Proc Natl Acad Sci USA* 106:13875–13879.
4. Müntzing A (1936) The evolutionary significance of autopolyploidy. *Hereditas* 21: 263–378.
5. Levin DA (1983) Polyploidy and novelty in flowering plants. *Am Nat* 122:1–25.
6. Ramsey J, Schemske DW (1998) Pathways, mechanisms and rates of polyploid formation in flowering plants. *Annu Rev Ecol Syst* 29:467–501.
7. Ramsey J, Schemske DW (2002) Neopolyploidy in flowering plants. *Annu Rev Ecol Syst* 33:589–639.
8. Stebbins GL (1950) *Variation and Evolution in Plants* (Columbia University Press, New York).
9. Lewis WH (1980) *Polyploidy: Biological Relevance*, ed Lewis WH (Plenum, New York), pp 103–144.
10. Johnston AW, Packer JG, Reese G (1965) *The Quaternary of the United States*, eds Wright HE, Frey DG (Princeton University Press, Princeton), pp 497–507.
11. Clausen J, Keck D, Hiesey WM (1945) Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autoploidy, with examples from the Madiinae. *Carnegie Institute of Washington, Publication No.* 564.
12. Levin DA (2002) *The Role of Chromosomal Change in Plant Evolution* (Oxford University Press, Oxford).
13. Parisod C, Holderegger R, Brochmann C (2010) Evolutionary consequences of autopolyploidy. *New Phytol* 186:5–17.
14. Soltis DE, Buggs RJA, Doyle JJ, Soltis PS (2010) What we still don't know about polyploidy. *Taxon* 59:1387–1403.
15. Keeler K (1992) Local polyploidy variation in the native prairie grass *Andropogon gerardii*. *Am J Bot* 79:1229–1232.
16. Felber-Girard M, Felber F, Buttler A (1996) Habitat differentiation in a narrow hybrid zone between diploid and tetraploid *Anthoxanthum alpinum*. *New Phytol* 133: 531–540.
17. Petit C, Bretagnolle F, Felber F (1999) Evolutionary consequences of diploid-polyploid hybrid zones in wild species. *Trends Ecol Evol* 14:306–311.
18. Segraves KA, Thompson JN (1999) Plant polyploidy and pollination: Floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. *Evolution* 53:1114–1127.
19. Husband BC, Schemske DW (2000) Ecological mechanisms of reproductive isolation between diploid and tetraploid *Chamerion angustifolium*. *J Ecol* 88:689–701.
20. Nuismer SL, Cunningham BM (2005) Selection for phenotypic divergence between diploid and autotetraploid *Heuchera grossulariifolia*. *Evolution* 59:1928–1935.
21. Thompson JD, Lumaret R (1992) The evolutionary dynamics of polyploid plants: Origins, establishment and persistence. *Trends Ecol Evol* 7:302–307.
22. Bretagnolle F, Lumaret R (1995) Bilateral polyploidization in *Dactylis glomerata* L. subsp. *lusitanica*: Occurrence, morphological and genetic characteristics of first polyploids. *Euphytica* 84:197–207.
23. Husband BC, Ozimec B, Martin SL, Pollock L (2008) Mating consequences of polyploid evolution in flowering plants: Current trends, and insights from synthetic polyploids. *Int J Plant Sci* 169:195–206.
24. Bretagnolle F, Thompson JD (1995) Tansley review no. 78. Gametes with the somatic chromosome number: Mechanisms of their formation and role in the evolution of autopolyploid plants. *New Phytol* 129:1–22.

25. Bretagnolle F (2001) Pollen production and spontaneous polyploidization in diploid populations of *Anthoxanthum alpinum*. *Biol J Linn Soc Lond* 72:241–247.
26. Ramsey J (2007) Unreduced gametes and neopolyploids in natural populations of *Achillea borealis* (Asteraceae). *Heredity* 98:143–150.
27. De Kovel CGF, De Jong G (2000) Selection on apomictic lineages of *Taraxacum* at establishment in a mixed sexual-apomictic population. *J Evol Biol* 13:561–568.
28. Clausen J, Keck D, Hiesey WM (1948) Experimental studies on the nature of species. III. Environmental responses of climatic races of *Achillea*. *Carnegie Institute of Washington, Publication No.* 581.
29. Ehrendorfer F (1973) New chromosome numbers and remarks on the *Achillea millefolium* polyploid complex in North America. *Osterr. Bot. Z.* 122:133–143.
30. Ramsey J, Robertson A, Husband B (2008) Rapid adaptive divergence in new world *achillea*, an autopolyploid complex of ecological races. *Evolution* 62:639–653.
31. Hiesey WM, Nobs M (1970) Genetic and transplant studies on contrasting species and ecological races of the *Achillea millefolium* complex. *Bot Gaz* 131:245–259.
32. Tyrl RJ (1975) Origin and distribution of polyploid *Achillea* (Compositae) in western North America. *Brittonia* 27:187–296.
33. Nobs M (1960) *Illustrated Flora of the Pacific States*, eds Abrams L, Ferris RS (Stanford University Press, California), pp 390–391.
34. Lawrence WE (1947) Chromosome numbers in *Achillea* in relation to geographic distribution. *Am J Bot* 34:538–545.
35. Hickman JC (1993) *The Jepson Manual: Higher Plants of California* (University of California Press, Berkeley).
36. Wiedemann AM, Dennis LR, Smith FH (1999) *Plants of the Oregon Coastal Dunes* (Oregon State University Press, Corvallis).
37. Soltis DE, et al. (2009) Polyploidy and angiosperm diversification. *Am J Bot* 96:336–348.
38. Van de Peer Y, Fawcett JA, Proost S, Sterck L, Vandepoele K (2009) The flowering world: A tale of duplications. *Trends Plant Sci* 14:680–688.
39. Coyne JA, Orr HA (2004) *Speciation* (Sinauer, Sunderland).
40. Rieseberg LH, Willis JH (2007) Plant speciation. *Science* 317:910–914.
41. Soltis PS, Soltis DE (2009) The role of hybridization in plant speciation. *Annu Rev Plant Biol* 60:561–588.
42. Udall JA, Wendel JF (2006) Polyploidy and crop improvement. *Crop Sci* 46:3–14.
43. Thompson JN, Merg KF (2008) Evolution of polyploidy and the diversification of plant-pollinator interactions. *Ecology* 89:2197–2206.
44. Maherali H, Walden AE, Husband BC (2009) Genome duplication and the evolution of physiological responses to water stress. *New Phytol* 184:721–731.
45. Guo Y-P, Saukel J, Mittermayr R, Ehrendorfer F (2005) AFLP analyses demonstrate genetic divergence, hybridization, and multiple polyploidization in the evolution of *Achillea* (Asteraceae-Anthemideae). *New Phytol* 166:273–289.
46. Clausen J, Keck D, Hiesey WM (1940) Experimental studies on the nature of species. I. Effect of varied environments on western North American plants. *Carnegie Institute of Washington, Publicaton No.* 520.
47. Tal M (1980) *Polyploidy: Biological Relevance*, ed Lewis WH (Plenum, New York), pp 61–75.
48. 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.
49. Rodríguez DJ (1996) A model for the establishment of polyploidy in plants. *Am Nat* 147:33–46.
50. Rowell DL (1994) *Soil Science: Methods and Applications* (Longman, New York).
51. Ter Braak CJF (1995) *Data Analysis in Community and Landscape Ecology*, eds Jongman RHG, ter Braak CJF, van Tongeren OFR (Cambridge University Press, Cambridge), pp 91–173.
52. Kenkel NC, Derksen DA, Thomas AG, Watson PR (2002) Review: Multivariate analysis in weed science research. *Weed Sci* 50:281–292.