PATHWAYS, MECHANISMS, AND RATES OF POLYPLOID FORMATION IN FLOWERING PLANTS

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ABSTRACT

Polyploidy is widely acknowledged as a major mechanism of adaptation and speciation in plants. The stages in polyploid evolution include frequent fertility bottlenecks and infrequent events such as gametic nonreduction and interspecific hybridization, yet little is known about how these and other factors influence overall rates of polyploid formation. Here we review the literature regarding polyploid origins, and quantify parameter values for each of the steps involved in the principal pathways. In contrast to the common claim that triploids are sterile, our results indicate that the triploid bridge pathway can contribute significantly to autopolyploid formation regardless of the mating system, and to allopolyploid formation in outcrossing taxa. We estimate that the total rate of autotetraploid formation is of the same order as the genic mutation rate (10^{-5}), and that a high frequency of interspecific hybridization (0.2% for selfing taxa, 2.7% for outcrossing taxa) is required for the rate of tetraploid formation via allopolyploidy to equal that by autopolyploidy. We conclude that the rate of autopolyploid formation may often be higher than the rate of allopolyploid formation. Further progress toward understanding polyploid origins requires studies in natural populations that quantify: (*a*) the frequency of unreduced gametes, (*b*) the effectiveness of triploid bridge pathways, and (*c*) the rates of interspecific hybridization.

INTRODUCTION

Polyploidy, defined as the possession of three or more complete sets of chromosomes, is an important feature of chromosome evolution in many eukaryote taxa. Yeasts, insects, amphibians, reptiles, and fishes are known to contain polyploid forms (100), and recent evidence of extensive gene duplication suggests that the mammalian genome has a polyploid origin (112). In plants, polyploidy represents a major mechanism of adaptation and speciation (24, 56, 95, 104, 120, 157, 159). It is estimated that between 47% and 70% of angiosperm species are polyploid (56, 110). Differences in ploidy have been observed among related congeners and even within populations of taxonomic species (24, 34, 56, 100, 156), and there is evidence that individual polyploid taxa may have multiple origins (154). These observations suggest that polyploid evolution is an ongoing process and not a rare, macroevolutionary event. Research in agricultural and natural systems indicates that polyploids often possess novel physiological and life-history characteristics not present in the progenitor cytotype (95, 104). Some of these new attributes may be adaptive, allowing a plant to enter a new ecological niche. Because plants of different ploidies are often reproductively isolated by strong post-zygotic barriers, polyploidy is also one of the major mechanisms by which plants evolve reproductive isolation (34, 56).

In spite of the prevalence and importance of polyploidy, the factors contributing to polyploid evolution are not well understood (165). Two critical stages of polyploid evolution can be identified: formation and establishment. To understand the process of polyploid formation requires information on the pathways, cytological mechanisms, and rates of polyploid formation. To assess the likelihood that a new polyploid will successfully establish requires information on the viability and fertility of new cytotypes, the extent of assortative mating and reproductive isolation within and between different cytotypes, and the ecological niche of new polyploids. Here we review the literature concerning polyploid formation to answer the following questions: (*a*) What are the primary pathways and mechanisms of polyploid formation? (*b*) What are the parameters for each of the steps involved in polyploid formation? (*c*) What are the numerical values reported for these parameters? and (*d*) What is the estimated rate of polyploid formation by each pathway?

One major motivation for this review is to synthesize the diverse literature on polyploid origins and thereby provide a resource for the development of future empirical and theoretical studies of polyploid evolution. To this end, we have tabulated data from many studies and made this information available on the *Annual Reviews* web site (http://www.annualreviews.org; see *Supplementary Materials*). We summarize these data throughout the text and identify the location of each database on the web site.

By necessity, many of the plants considered in this review are agricultural or horticultural cultivars and their wild relatives, as well as taxa widely used in classical genetic studies (e.g. *Oenothera* and *Datura*). We believe that the studies reviewed here provide insights into the process of polyploid formation in natural populations, but caution that further research in natural populations is needed to test our findings. Our survey draws from a wide range of plant taxa, but because of the limited number of studies, we do not interpret our results in a phylogenetic context.

In this chapter, $2n$ refers to the somatic chromosome number and n to the gametic chromosome number regardless of the degree of polyploidy, while x is the most probable base number. This gives the following cytological designations: diploids ($2n = 2x$), triploids ($2n = 3x$), tetraploids ($2n = 4x$), etc. In describing crosses within and between cytotypes, the maternal parent is always listed first.

MECHANISMS OF POLYPLOID FORMATION

Several cytological mechanisms are known to induce polyploidy in plants. Somatic doubling in meristem tissue of juvenile or adult sporophytes has been observed to produce mixoploid chimeras (2, 66, 82, 128, 153). For example, *Primula kewensis*, one of the first described allopolyploids, originated from fertile tetraploid shoots on otherwise sterile diploid F_1s of *P. floribunda* × *P. verticellata* (127). Similarly, a tetraploid shoot was observed on a diploid F_1 hybrid between *Mimulus nelsoni* and *M. lewisii* (66), and in wounded ("decapitated") tomato plants (82). Somatic polyploidy is known to be common in many non-meristematic plant tissues (30, 31). For example, normal diploid *Vicia faba* contains tetraploid and octoploid cells in the cortex and pith of the stem (26). Such polyploid cells occasionally initiate new growth, especially in wounds or tumors, and are a potentially important source of new polyploid shoots (30, 31, 99). The frequency of endopolyploidy, and the relative likelihood of polyploid formation from different endopolyploid tissues, are not well known.

Somatic doubling can also occur in a zygote or young embryo, generating completely polyploid sporophytes. This phenomenon is best described from heat shock experiments in which young embryos are briefly exposed to high temperatures (43, 140). Corn plants exposed to $40°C$ temperatures approximately 24 h after pollination produced 1.8% tetraploid and 0.8% octoploid seedlings (140). Polyploid seedlings are also known to arise from polyembryonic ("twin") seeds at a high frequency (122, 176), but it is now believed that such polyploids are generally of meiotic rather than somatic origin (29). In general, little is known about the natural frequency of somatic doubling in plants nor of the effects of interspecific hybridization on its occurrence.

A second major route of polyploid formation involves gametic "nonreduction," or "meiotic nuclear restitution," during micro- and megasporogenesis. This process generates unreduced gametes, also referred to as "$2n$ gametes," which contain the full somatic chromosome number (see reviews in 19, 63). The union of reduced and unreduced gametes, or of two $2n$ gametes, can generate polyploid embryos. As will be described in detail below, $2n$ gametes have been identified in many plant taxa. Polyspermy, the fertilization of an egg by more than one sperm nucleus, is known in many plant species (172), and has been observed to induce polyploidy in some orchids (59). However, it is generally regarded as an uncommon mechanism of polyploid formation (56).

Distinguishing between somatic doubling and $2n$ gametes as mechanisms of polyploid formation requires a system of genetic markers and a detailed knowledge of the cytological mechanism of gametic nonreduction, which are seldom available. There is, however, strong circumstantial evidence that $2n$ gametes are often involved in polyploid formation. The parents of spontaneous polyploids have, upon cytological analysis, commonly been found to produce $2n$ gametes (18, 21, 24, 46, 52, 55, 83, 90, 94, 101, 166, 168, 173). Conversely, plants known to produce $2n$ gametes can be crossed to produce new polyploids (18, 37, 81, 136, 138). In many cases, spontaneous polyploids have cytotypes that appear to have been formed by the union of reduced and unreduced gametes (21, 67, 68, 84, 125) rather than by somatic mutation, which generally only doubles the base chromosome number (e.g. $4x$ to $8x$) (30, 66, 82, 127). For example, Navashin (125) found triploids and pentaploids in the progeny of open-pollinated diploid *Crepis capillaris*, and these appear to have been produced by the union of reduced (n) and unreduced ($2n$ and $4n$) gametes. Similarly, triploids generated by backcrossing diploid hybrid *Digitalis ambigua* × *purpurea* are thought to have arisen from unreduced gametes produced by this interspecific hybrid (21). Because nonreduction appears to be the major mechanism of polyploid formation (19, 63, 165), we focus on the role of $2n$ gametes in polyploid origins. It is clear, however, that much research remains to determine the relative roles of the various cytological mechanisms of polyploid formation in natural populations.

Auto- and Allopolyploidy: An Evolving Terminology

Kihara & Ono (86) first described two distinct types of polyploids: "autopolyploids," which arise within populations of individual species, and "allopolyploids," which are the product of interspecific hybridization. Because chromosome pairing behavior was believed to be a reliable indicator of chromosome homology, early workers emphasized the frequency of multivalent formation at synapsis as a criterion for distinguishing auto- and allopolyploidy (32, 120). It was subsequently recognized that some polyploids of known hybrid origin

exhibit multivalent pairing, while bivalent formation is prevalent in some non-hybrid polyploids (24, 156, 157). The term "segmental allopolyploid" was thus coined to denote polyploids of hybrid origin that possess chromosome pairing characteristics of autopolyploids, while "amphiploid" was used to indicate all polyploids that combine the chromosome complements of distinct species (24, 157). The term "autopolyploid" was reserved for polyploids that arose within single populations or between ecotypes or races of a single species (24, 56). Although this terminology is recognized by many students of polyploidy (56, 165), several confusing aspects remain. For example, there is considerable variation in the criteria used to delimit related taxa as "species." Moreover, some authors reserve the term allopolyploidy for hybrid polyploid derivatives of species that are largely reproductively isolated by barriers of hybrid sterility, because such species are more likely to differ in chromosome structure and pairing and to generate polyploids that behave cytogenetically as "true" allo-ploids (24, 160). Also, it is to be expected that some interpopulation polyploids may represent a class of polyploidy intermediate between auto- and allopoly-ploidy. Because of these and other difficulties, several alternate terminologies have been suggested. Jackson (71) proposed that the terms auto- and allopoly-ploidy be used in their original, cytological meaning (32, 120)—that autopoly-ploids exhibit multivalent pairing while allopolyploids do not—and developed statistical criteria for distinguishing these types of polyploids (72). Lewis (99) used "intraspecific" and "interspecific" polyploidy to distinguish polyploids that are morphologically distinguishable from those that are not, and considered these terms to correspond roughly to "autopolyploidy" and "allopolyploidy," respectively.

We believe that the primary criterion for classifying a polyploid is its mode of origin. We use the term "autopolyploid" to denote a polyploid arising from crosses within or between populations of a single species, and "allopolyploid" to indicate polyploids derived from hybrids between species, where species are defined according to their degree of pre- and/or post-zygotic isolation (biological species concept). We consider polyploids arising from hybridization between species with minor aneuploid differences (dysploidy) to be allopolyploids, following Clausen et al (24). Considerable differences in the mechanisms and rates of polyploid formation within "types" of autopolyploid and allopoly-ploid systems may exist. In particular, the frequency of meiotic irregularity and spontaneous polyploid production may differ between hybrids of recently and anciently diverged taxa.

Pathways of Polyploid Formation

Several different pathways of both auto- and allopolyploid formation have been described. In this section, we identify the major routes to polyploid formation

and highlight examples in which one or more steps in the pathway have been directly observed.

AUTOTETRAPLOID, TRIPLOID-BRIDGE   Triploids are formed within a diploid population, and backcrossing to diploids, or self-fertilization of the triploid, produces tetraploids. For example, 1% tetraploid progeny were obtained by backcrossing a spontaneous triploid clone of *Populus tremula* to a diploid (15). Similarly, a small number of tetraploid progeny were obtained from triploid apple varieties that had themselves originated as spontaneous polyploids (14). Although all the steps in this pathway have rarely been observed in their entirety, the individual mechanisms are well substantiated. Triploids have often been observed in diploid populations (41, 47, 69, 143, 168), and it is generally believed that these are produced by the union of reduced (n) and unreduced ($2n$) gametes. Studies of such spontaneous triploids, as well as triploids produced by crossing diploids and tetraploids, indicate that many of the gametes produced by autotriploids are not functional, because they possess aneuploid, unbalanced chromosome numbers. However, triploids generate small numbers of euploid (x, $2x$) gametes (12, 40, 44, 87, 91, 148, 149) and can also produce $3x$ gametes via nonreduction (12, 91, 117). Autotriploids can produce tetraploids by self-fertilization or backcrossing to diploids (40, 44, 74, 174, 179).

AUTOTETRAPLOID, ONE-STEP   Tetraploids are formed directly in a diploid population by the union of two unreduced ($2n$) gametes or by somatic doubling. For example, Einset (47) found a small fraction (4.39×10^{-4}) of tetraploid seedlings while cytotyping the progenies of open-pollinated diploid apple varieties. Tyagi (168) crossed clones of *Costus speciosus* that were observed to produced some $2n$ pollen, and recovered a small number of tetraploid seedlings. This process has been observed in several other taxa (18, 69, 81, 85, 89, 122).

ALLOTETRAPLOID, TRIPLOID-BRIDGE   Hybrid triploids are formed by diploids in the F_1 or F_2 generation of interspecific crosses, and self-fertilization or backcrossing to diploids produces allotetraploids. For example, Müntzing (119) crossed *Galeopsis pubescens* and *Galeopsis speciosa* to generate a highly sterile, diploid F_1 hybrid. One of the 200 F_2 progeny was found to be triploid, and a backcross of this plant to *G. pubescens* formed a single viable seed, which was tetraploid (119). Similarly, Skalińska (152) found a single triploid plant in the F_1 progeny of a cross between *Aquilegia chrysantha* and *Aquilegia flavellata*. Selfing this triploid produced a small number of F_2 progeny, two thirds of which were tetraploid. Allotriploids have commonly been observed in the F_2 generation produced by backcrossing or selfing interspecific F_1 hybrids (21, 24, 94, 101, 118, 137), and in the F_1 generation by the union of reduced and unreduced gametes from the parent genotypes (27, 60, 67,

152). Studies of such spontaneous allotriploids, and of allotriploids obtained by crossing different diploid and tetraploid species, indicate that the production of diploid gametes (8, 54, 152, 169) and nonreduction (45, 70, 90, 162) enable allotriploids to produce allotetraploids by selfing or backcrossing (67, 80, 118, 152, 167).

ALLOTETRAPLOID, ONE-STEP   Allotetraploids are formed directly from diploids in the F_1 or F_2 generation of interspecific crosses. For example, 90% of the F_2 progeny of *Digitalis ambigua* and *Digitalis purpurea* were tetraploid (21). Half of the F_2 progeny of *Allium cepa* and *Allium fistolium* were tetraploid or hypotetraploid (i.e. $4x - 1$) (94), and 2% of the F_1 progeny of *Manihot epruinosa* × *glaziovii* were tetraploid (60). There are many other examples of tetraploids being produced in one step by F_1 interspecific hybrids (1, 21, 24, 46, 55, 66, 83, 128, 137), or in the F_1 generation of an interspecific cross (60, 79, 92, 169). Other important pathways involve the evolution of ploidy levels above tetraploidy.

HIGHER PLOIDY, ONE-STEP   Within a polyploid population, the union of reduced and unreduced gametes generates a new cytotype of higher ploidy. For example, 2% hexaploid cytotypes were recovered from the progeny of open-pollinated autotetraploid *Beta vulgaris*, apparently from the union of reduced ($2x$) and unreduced ($4x$) gametes (68). Similarly, 1% of the progeny of tetraploid alfalfa were found to be hexaploid (16). There is circumstantial evidence of autohexaploid formation in tetraploid populations in several other systems (23, 42). New odd-ploidy cytotypes could also be produced by this mechanism. For example, it has been suggested that unreduced gamete production in hexaploid *Andropogon gerardii* generated a $9x$ cytotype, which is now widely distributed (130).

ALLOPOLYPLOIDY, VIA HYBRIDIZATION OF AUTOPOLYPLOIDS   Hybridization between distinct autopolyploids directly produces allopolyploids. For example, crosses between autotetraploid *Lycopersicon esculentum* and autotetraploid *Lycopersicon pimpinellifolium* produced a fertile allotetraploid *Lycopersicon* that was identical to the allotetraploid made by doubling the diploid F_1 hybrid (103). An allotetraploid *Tradescantia* was produced by crossing autotetraploid forms of *T. canaliculata* and *T. subaspera* (4). It has repeatedly been found that the post-zygotic barriers that isolate diploid taxa break down in autopolyploids, so that interspecific hybrids are formed easily (23, 65). Not surprisingly, there may be extensive intergradation among polyploids, while diploid taxa remain morphologically distinct (65, 98).

ALLOPOLYPLOIDY, VIA HYBRIDIZATION OF DIFFERENT CYTOTYPES   Hybridization between different cytotypes (which may be of auto- or allopolyploid origin)

generates intermediates of odd-ploidy, which subsequently produce new even-ploidy cytotypes. For example, a high frequency ($\sim$81%) of allohexaploids was generated by crossing triploid hybrids of *Nicotiana paniculata* (2*x*) and *Nicotiana rustica* (4*x*) (90). A similar process has been observed in many other systems (24, 25, 90, 166, 173).

Second-Generation Polyploids

The production of later-generation polyploids can be achieved through a variety of pathways. For example, a new self-compatible tetraploid can self to produce tetraploid offspring. For outcrossing taxa, second-generation tetraploids can be produced by matings between independently produced tetraploids. Alternatively, backcrossing to the diploid progenitor can produce triploids (18, 73, 76), which contribute to further tetraploid formation by crossing to either diploids (44, 80, 152, 170, 179) or tetraploids (40, 139, 152, 171, 174). Later-generation tetraploids can also be produced by backcrosses of tetraploids to diploids that produce unreduced gametes (18, 51, 69, 178). Clearly, the frequency and cytotype composition of later-generation polyploids will depend on factors such as the mating system and the degree of pre- and post-zygotic reproductive isolation between cytotypes (50, 144). There is a need for further empirical and theoretical research on these and other issues related to polyploid establishment.

UNREDUCED GAMETES AND THE ORIGIN OF NEW POLYPLOIDS

Unreduced gametes are believed to be a major mechanism of polyploid formation (19, 64). Both 2*n* pollen and 2*n* eggs have been observed in hybrid and non-hybrid agricultural cultivars and natural plant species (11, 13, 24, 36, 37, 83, 101, 116, 136, 142, 147, 151, 163, 164, 177). Unreduced pollen grains can often be identified by size, as they typically have a diameter 30–40% larger than that of reduced pollen (78, 168, but see 101, 105), and the distribution of pollen size in plants known to produce 2*n* pollen is often bimodal (131, 168). Unreduced female gametophytes can sometimes be identified by size (161), but more often the frequency of 2*n* gametes is indirectly estimated using controlled, inter-ploidy crosses in plants with very strong interploidy crossing barriers. The progeny generated are usually the products of 2*n* gametes (3, 36; see below). Little correlation has been observed between the production of 2*n* pollen and 2*n* eggs (36, 134, 142, 164, but see 147).

Meiotic aberrations related to spindle formation, spindle function, and cytokinesis have been implicated as the cause of 2*n* gamete production in nonhybrid crop cultivars (19). For example, a parallel spindle orientation at anaphase II

results in the reconstitution of diploid nuclei in microsporogenesis, and premature cytokinesis that immediately follows the first meiotic division creates diploid nuclei that never undergo a second meiotic division (116). The cytological causes of nonreduction in hybrids are less well studied. Often, poor chromosome pairing in F_1 hybrids leads to asynapsis at the first meiotic division, and a single "restitution" nucleus containing the full somatic chromosome number forms in the spore mother cell (55, 83, 166, 173). Other cytological mechanisms related to cytokinesis are also known to produce $2n$ gametes in interspecific hybrids (64). The timing and type of cytological anomaly producing $2n$ gametes affects both the level of genic heterozygosity and the yield of polyploid cultivars (114). Unfortunately, there are few data on the cytological origins of $2n$ gametes in natural systems. Identifying the origins of unreduced gametes is complicated, because different individuals in the same species often produce $2n$ gametes by different cytological mechanisms, and more than one mechanism may operate within an individual plant (134, 177).

What Is the Frequency of Unreduced Gametes?

The frequency of $2n$ gametes determines the rate of new polyploid formation, as well as the types of polyploids being produced, and is therefore critical for understanding polyploid formation. We summarized the observed frequencies of $2n$ pollen in hybrid and nonhybrid systems, excluding those selected for their tendency to produce $2n$ gametes.

The mean frequency of $2n$ gametes found in studies of hybrids (27.52%) was nearly 50-fold greater than that in nonhybrids (0.56%), and this difference was significant (Mann-Whitney U test, $P < 0.001$) (web Table 1; 8 web Tables are located at www.AnnualReviews.org). This result is consistent with the qualitative impressions of Harlan & de Wet (63). Because interspecific hybrids often experience severe meiotic irregularities involving poor chromosome pairing and non-disjunction, the "reduced" gametes produced by hybrids often possess unbalanced, aneuploid cytotypes, and are thus inviable (22, 52). This suggests that the effective frequency of $2n$ pollen in hybrid systems may be even higher than estimated here.

The few existing data for $2n$ eggs suggest that the natural frequency of nonreduction is similar in megasporogenesis and microsporogenesis. The mean frequency of $2n$ eggs in a sample of approximately 100 field-collected individuals of *Dactylis glomerata* was 0.49% (36), while the frequency of $2n$ pollen in a similar collection of individuals was found to be 0.98% (105). The mean frequency of $2n$ eggs was 0.06% in *Trifolium pratense* (136) and 0.09% in maize (3). We know of no published reports on the frequency of $2n$ eggs in interspecific hybrids.

Polyploid Formation Is Facilitated by a Breakdown in Self-Incompatibility

The "effective" frequency of 2n pollen in plants with gametophytic self-incompatability may be increased by the breakdown of incompatibility in diploid pollen produced by either reduction division in established tetraploids, or nonreduction in diploids. This tendency, which appears to be related to genic interactions in diploid pollen grains (20, 96, 97), may allow self-pollination by 2n pollen, leading to polyploid formation. For example, Marks (108) obtained some polyploids by selfing diploid self-incompatible *Solanum*. Lewis (96) found only triploids in the selfed progeny of self-incompatible strains of *Pyrus* producing 2n pollen, and described this phenomenon as the "incompatibility sieve" for polyploid formation. This mechanism could contribute to polyploid formation where abiotic or biotic factors increase the frequency of self pollen deposition.

Genetic Factors Influence the Rate of Unreduced Gamete Production

Bretagnolle & Thompson (19) provide an exhaustive review of the genetic basis of 2n gamete production in nonhybrid crop species, and we provide only a brief summary here. Plant populations often possess heritable genetic variation for the capacity to produce 2n gametes, as illustrated by a rapid response to selection for 2n gamete production in crop cultivars (135, 164). For example, the mean frequency of 2n pollen increased from 0.04% to 47% in three generations of selection on *Trifolium pratense*, giving a realized heritability of 0.50 (135). In *Medicago sativa*, selection experiments on 2n pollen and 2n egg production gave realized heritabilities of 0.39 and 0.60, respectively (164). Meiotic analysis of progeny derived from crosses between plants differing in their level of 2n gamete production indicate that this phenotype can be under strong genetic control and is often determined by a single locus (115, 142, 147).

Why Is There Genetic Variation for Unreduced Gamete Production?

Because different cytotypes are typically reproductively isolated, 2n gametes do not contribute to the gene pool of their progenitor cytotype. Thus, we expect strong selection against 2n gamete production, and it is perhaps surprising to sometimes find high heritabilities for this trait. As yet, there is insufficient information to determine if the frequency of genes influencing 2n gamete production is different from that expected by mutation-selection balance. Polyploidy often occurs in perennial taxa capable of vegetative reproduction (58, 155).

Characters related to sexual reproduction may be under relaxed selection in these systems, resulting in a potentially higher frequency of $2n$ and nonfunctional gametes. This hypothesis is supported by the observation that many of the taxa in which $2n$ gamete production has been documented are perennials with means of vegetative propagation (9, 105, 131). Another possible mechanism contributing to $2n$ gamete production is that the cytological abnormalities leading to non-reduction are the pleiotropic effect of genes with other, perhaps beneficial, effects.

Environmental Factors Can Affect the Frequency of Unreduced Gametes

Several researchers have found that $2n$ pollen production is stimulated by environmental factors such as temperature, herbivory, wounding, and water and nutrient stress. Temperature, and especially variation in temperature, have particularly large effects (11, 38, 96, 111, 151). Belling (11) observed a dramatic increase in $2n$ pollen production in field and greenhouse cultures of *Strizolobium* sp., *Datura stramonium*, and *Uvularia grandiflora* following aberrant cold spells. Potato genotypes selected for the tendency to undergo gametic non-reduction had approximately twice the mean frequency of $2n$ pollen in a coastal field as in a greenhouse, an effect attributed to the temperature differences of the two environments (111). Ramsey & Schemske (unpublished data) found that the frequency of $2n$ pollen in randomly selected *Achillea millefolium* plants reared in a temperature-cycling growth chamber was approximately six times that in the natural population from which the study plants had been sampled.

Plant nutrition, herbivory, and disease may also affect $2n$ gamete production. Grant (55) found that the rate of polyploid production per flower in F_1 *Gilia* hybrids grown in low-nutrient conditions was almost 900 times greater than that of plants grown in high-nutrient conditions, a result attributed to poor pairing at meiosis in the former treatment. However, the higher level of polyploid production per flower in the low-nutrient treatment was partially offset by a much lower flower number, such that the number of polyploids produced per plant was only seven-fold greater. Kostoff (88) and Kostoff & Kendall (89) described an effect of gall mites and tobacco mosaic virus on $2n$ pollen formation.

Many of the environmental factors known to influence $2n$ gamete production are experienced by plants in their natural habitats. This suggests that natural environmental variation, as well as large-scale climate change, could substantially alter the dynamics of polyploid evolution. The high incidence of polyploidy at high latitudes, high altitudes, and recently glaciated areas may be related to the tendency of harsh environmental conditions to induce $2n$ gametes and polyploid formation (23, 151).

TRIPLOIDS: FORMATION, MEIOSIS, FERTILITY, AND PROGENY

The evolution of tetraploidy may proceed directly from diploids via the union of two $2n$ gametes, or in two steps via a triploid bridge (19). Because the probability of the union of two $2n$ gametes is expected to be very low, it has been hypothesized that triploids usually play a role in the evolution of tetraploids (39, 63). However, the low fertility of triploids, coupled with the existence of cytological barriers that may prevent or limit triploid formation by diploids, may restrict the role of triploids (19, 150). We review components of the triploid bridge pathways, including the likelihood of triploid formation via $2n$ gametes, triploid fertility and meiotic behavior, and the cytotype composition of the progeny produced by triploid parents.

How Effective Are Unreduced Gametes in Triploid Formation?

In most flowering plants, fertilization of the egg by a sperm nucleus is accompanied by fusion of the other sperm nucleus with two haploid polar nuclei in the female gametophyte to form the triploid endosperm that functions to nourish the $2x$ embryo. In polyploids, the process proceeds in an analogous fashion, but the ploidies of all tissues are proportionately increased. Crosses between diploid and tetraploid plants often fail because intercytotype hybrid seed development does not proceed normally, and nonviable seeds are produced. The difficulty of obtaining viable triploid seeds by diploid-tetraploid and tetraploid-diploid crosses has been termed the "triploid block" (108). Barriers to intercytotype hybridization have been observed at higher ploidy levels, but are not well described. Viable seeds produced by crosses between diploids and tetraploids are often tetraploid and result from unreduced gametes produced by the diploid parent.

Abnormalities in the growth and structure of the endosperm have often been implicated as the source of triploid block (28, 49, 113, 178; see reviews in 62, 175). The ratios between the embryo, endosperm and/or maternal tissue, as well as the maternal:paternal ploidy ratio in the endosperm, are all altered in $2x \times 4x$ and $4x \times 2x$ crosses, and it has been suggested that normal seed development depends on these ploidy ratios. Müntzing (123) hypothesized that proper function requires an embryo:endosperm:maternal tissue ratio of 2:3:2, while others have suggested that it is the 2:3 ratio of the embryo to endosperm that is critical for proper seed development (175). An alternate explanation is that the maternal:paternal ploidy ratio in the endosperm, irrespective of the ploidy of the embryo or maternal tissue, determines seed viability (129). This has been described as the imprinting hypothesis (61, 102), and supporting evidence

is provided by several studies showing that a 2:1 ratio of the maternal:paternal genomes in the endosperm is required for normal seed development (77, 102). Johnston et al (77) proposed a modification of this hypothesis to account for the anomalous findings that viable seed is produced in some systems where the 2:1 ratio is violated, but not in others where the 2:1 ratio is met. Their endosperm balance number hypothesis suggests that seed development is affected by the effective maternal:paternal ploidy ratio of the endosperm, which may not always reflect the actual ploidy composition (77). Although the genetic mechanisms responsible for interploidy crossing barriers remain an open area of investigation, there is general agreement that the ploidies of an embyro and/or its associated endosperm are the critical factors influencing successful seed development.

In diploids, fertilization of a reduced egg by an unreduced sperm nucleus will generate the same embryo:endosperm ploidy ratio (3:4) as a $2x \times 4x$ cross, and the union of an unreduced egg and a reduced sperm nucleus will form the ploidy ratio (3:5) of a $4x \times 2x$ cross [most described mechanisms of unreduced egg formation involve nonreduction in the megaspore mother cell, thus producing gametophytes with unreduced polar and egg nuclei (142, 147, 177, but see 29)]. Similarly, seed from a $2x \times 4x$ cross contains endosperm with the same maternal:paternal ratio (2:2) of seeds produced by the union of reduced eggs and unreduced pollen, while seed from a $4x \times 2x$ cross contains endosperm with the same maternal:paternal ratio (4:1) of seeds produced by the union of unreduced eggs and reduced pollen. Note that crosses in both directions violate the normal ($2x \times 2x$) embryo:endosperm ratio of 2:3 and the endosperm maternal:paternal ratio of 2:1. Irrespective of the cytological cause of triploid block, the success of crosses involving diploid reduced gametes from tetraploids and haploid reduced gametes from diploids should parallel that of crosses involving diploid unreduced gametes from diploids and reduced gametes from diploids. Here we use this approach to evaluate the likelihood of triploid formation via $2n$ gametes. We surveyed the literature for data on crosses between autotetraploids and their progenitor diploids. We excluded studies of naturally occurring polyploids because genic differences arising after autopolyploid formation could contribute to crossing barriers. Our review focuses on studies of autopolyploids because there are too few data on crossing success in allopolyploids.

No viable triploid seed production was observed in 13 of the 19 studies of $2x \times 4x$ crosses, or in 7 of the 17 studies of $4x \times 2x$ crosses (web Table 2 located at www.AnnualReviews.org). These data indicate that complete triploid block is present in many taxa, and that the possibility of triploid formation may differ for $2n$ pollen and eggs. In the 11 studies reporting viable triploid production in $2x \times 4x$ and/or $4x \times 2x$ crosses, 10 found that more viable triploid seeds were generated by $4x \times 2x$ crosses, while in only a single study

was higher triploid production from $2x \times 4x$ crosses observed. This difference is statistically significant (Paired sign test, $P < 0.05$).

We calculated an index of triploid block via $2n$ gametes using studies in which data on intracytotype crosses were available. For diploid pollen, this is

$$1 - \frac{k_{(2\times4).3}}{k_{(2\times2)}}, \qquad\qquad 1.$$

where $k_{(2\times4).3}$ is the viable triploid seed production from $2x \times 4x$ crosses, and $k_{(2\times2)}$ is the viable seed production from $2x \times 2x$ crosses (assumed to include only diploid seed). The corresponding likelihood of triploid formation from $2n$ eggs is

$$1 - \frac{k_{(4\times2).3}}{k_{(2\times2)}}, \qquad\qquad 2.$$

where $k_{(4\times2).3}$ is the viable triploid seed production from $4x \times 2x$ crosses, and $k_{(2\times2)}$ is defined as above. For both calculations, values greater than 0 indicate a triploid block, and a value of 1.0 indicates a complete block.

In the nine studies involving $2x \times 4x$ crosses, the mean block via diploid pollen was 0.952 (range 0.57 to 1.0), and of the eight studies of $4x \times 2x$ crosses, the mean block via diploid eggs was 0.801 (range 0.34 to 1.0), a difference that is marginally significant (Wilcoxon Sign Rank test, $P = 0.07$). Together, these data demonstrate there is generally a large barrier to triploid formation via unreduced gametes, but that the barrier is often not complete. A direct estimate of triploid block would compare observed and expected triploid production in crosses between diploids producing $2n$ gametes. In *Dactylis glomerata*, this approach gave an overall block of 0.98 (18), which is similar to the mean value we report here.

The observed reciprocal differences in intercytotype crossing success suggest that unreduced eggs are more effective in polyploid formation than are unreduced pollen. This is consistent with the finding that spontaneous triploids in well-studied genetic systems arise via nonreduction in female parents (17, 27). Reciprocal differences in interploidy crossing success may be a consequence of the embryo:endosperm and endosperm maternal:paternal ploidy ratios, which differ with the direction of the cross (62, 175). By cytotyping the embryos and endosperm of *Citrus* seeds resulting from intracytotype and intercytotype crosses of parents producing both reduced and unreduced gametes, Esen & Soost (49) demonstrated that seeds with embryo:endosperm ratios of 2:3, 3:5, 4:6, and 6:10 were viable, while those with 3:4, the expected ratio resulting from the union of unreduced pollen with reduced eggs, were not. Using a meiotic mutant that generated endosperm of varying ploidy, Lin (102) showed an effect of endosperm genome composition on seed viability in $2x \times 2x$ and $2x \times 4x$

crosses in maize. Another possible cause of reciprocal differences is that some mutations in megasporogenesis create embryo sacs containing nuclei of varying ploidy, and thus triploid embryos produced by these megagametophytes can be accompanied by normal, functional triploid endosperm (29). These mutations are a cause of polyembryony (29) and may be an important route of viable triploid formation. Regardless of its cause, the possibility that unreduced eggs are of primary importance in triploid formation seems significant in light of the fact that nearly all studies of gametic nonreduction have focused on unreduced pollen (24, 46, 101, 168, 173).

Several taxa with low triploid block have atypical endosperm characteristics (62, 178). In *Oenothera*, only a single polar nucleus is involved in the formation of a diploid endosperm, and viable triploid seeds are easily produced by $2x \times 4x$ crosses (62). In *Populus*, mature seeds have no endosperm, and seed development is very rapid; viable seed set in $2x \times 4x$ crosses is 80% of the $2x \times 2x$ yield (75). These observations suggest a possible relationship between endosperm characteristics, the strength of triploid block, and polyploid formation. Plant families such as the Asteraceae, Crassulaceae, Onagraceae, Rosaceae, and Salicaceae that lack endosperm in mature seeds have a high incidence of polyploidy.

Triploids Generate Some Euploid Gametes, and Are Often Semi-Fertile

The reduction division in triploids is expected to generate aneuploid gametes with half the triploid chromosome number, or $3x/2$. However, the possession of an unmatched complement of chromosomes leads to the formation of multivalents and univalents during pairing, and subsequent irregularities during disjunction can create varied chromosome assortments (33).

We surveyed the literature to examine the cytotype composition of pollen produced by triploids of both hybrid and nonhybrid origin, as indicated by examination of anaphase I, metaphase II, and anaphase II in pollen mother cells, or the first postmeiotic mitosis in maturing pollen. In the 26 studies examined, the most common modal pollen chromosome number was $3x/2$ (17 studies), followed by $3x/2 - 1$ (5 studies; web Table 3). The tendency to produce aneuploid pollen is similar in auto- and allotriploids, so in the remaining analyses we consider both triploid types together. Figure 1 shows the average frequency of haploid, diploid, triploid, and the most common aneuploid cytotypes in pollen produced by triploids ($n = 25$ studies; web Table 3 located at www.AnnualReviews.org). Low mean frequencies were found for haploid (3%) and diploid (2%) pollen relative to the frequency of the common aneuploid class $3x/2$ (34%). Rare euploid gametes are formed when the separation of multivalents and unpaired chromosomes in a spore mother cell is so unequal
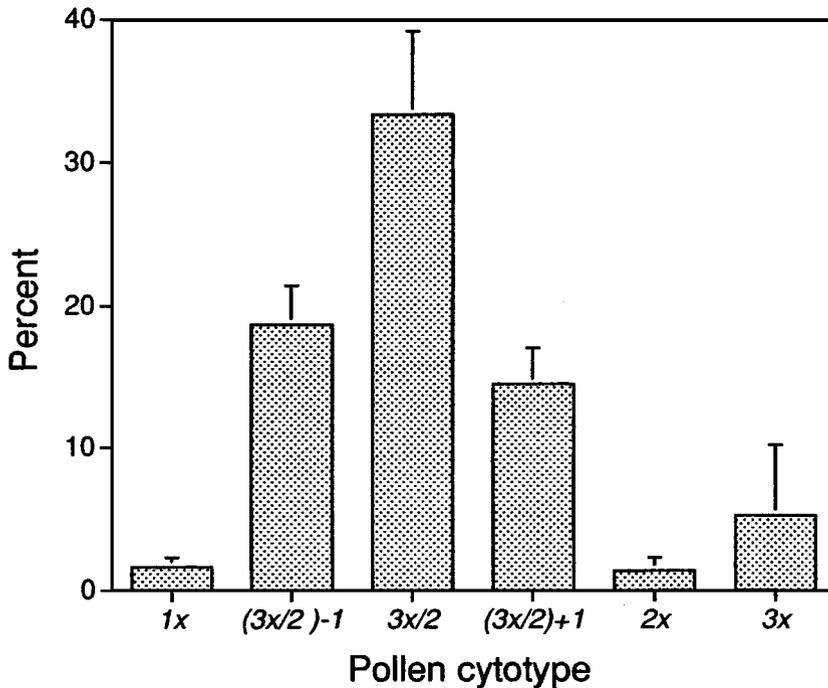
Figure 1   Frequency of euploid (1x, 2x, 3x) and common aneuploid ($3x/2$, $3x/2 - 1$, $3x/2 + 1$) cytotypes in pollen produced by hybrid and nonhybrid triploids, as determined by investigation of pollen mitoses as well as metaphase and anaphase of pollen mother cells. Data from web Table 1.

that haploid-diploid chromosome assortments are produced at the first meiotic division (12, 44, 149, 152). Triploid pollen, the result of gametic nonreduction (12, 45, 70, 91), was also observed at a low mean frequency (5.2%; $n = 9$ studies). Together, these analyses suggest that triploids produce mostly aneuploid classes of pollen. Unfortunately, there are relatively few studies of megasporogenesis to complement the data on microsporogenesis. Satina & Blakeslee (148) observed a modal egg chromosome number of $3x/2 - 2$ in triploid *Datura stramonium*, with 7% haploid and 1% diploid complements. These values are similar to those observed for pollen.

Triploids are often expected to be sterile because of their meiotic irregularities and high frequency of aneuploid gametes. However, in a survey of the literature, we found a mean pollen fertility of 31.9% (range 0–97; web Table 4 located at www.AnnualReviews.org). The mean fertility of autotriploids (39.2%, $n = 23$ studies) was greater than that of allotriploids (23.7%, $n = 18$ studies), but this

difference was not significant (Mann-Whitney U test, $P = 0.17$). For those studies with data on both pollen cytotype and fertility (web Table 4 located at www.AnnualReviews.org), there was a significant positive correlation between the frequency of euploid (x and $2x$) pollen and pollen fertility (Spearman Rank Correlation, $r = 0.63$, $P < 0.05$, $n = 11$), suggesting that euploid pollen contribute disproportionately to overall pollen fertility (the frequency of $3x$ pollen was not included in this analysis because most studies quantifying gametic nonreduction did not provide data on the frequency of $1x$ and $2x$ pollen). Further evidence that triploids are often semifertile comes from the few studies that have examined the relative fertility of crosses involving triploids. The available data suggest that some viable progeny are typically obtained from $2x \times 3x$, $3x \times 2x$, and $3x \times 3x$ crosses, and that crossing success may vary with the direction of the cross (51, 126, 152, 170).

New Polyploids Can Be Generated Through a Triploid Bridge

The cytotypes of the progeny derived from triploid crosses are often different from what might be expected from triploid meiotic behavior. Figure 2 illustrates this phenomenon in allotriploid *Aquilegia chrysantha* × *flavellata* ($3x = 21$) and autotriploid *Zea mays* ($3x = 30$). In both cases, pollen chromosome numbers had an approximately normal distribution (Figure 2*a, b*), with modes corresponding to the "expected" aneuploid value of $3x/2$. However, the cytotype distribution of the progeny differed significantly from the expected distributions calculated from the chromosome number distribution in microsporogenesis for self (Figure 2*c, d*) and backcross (not shown) progeny (Kolmogorov-Smirnov One-Sample test, $P < 0.01$). The selfed progeny in both studies had a bimodal cytotype distribution; in *Aquilegia*, most of the offspring were fully tetraploid (Figure 2*c*), while the modes in *Zea* were aneuploid (Figure 2*d*). These results suggest that gametes with cytotypes near the modal class of $3x/2$ do not function as well as other gametes, especially compared to those with euploid or near-euploid cytotypes.

We surveyed the literature to examine the frequency of polyploid cytotypes in the progeny of triploids. Auto- and allotriploids produced similar progeny cytotypes, so we combined them for the following analyses. Figure 3 illustrates the mean frequency of several euploid and aneuploid cytotypes resulting from $2x \times 3x$, $3x \times 2x$, $3x \times 3x$, $3x$ self, $3x \times 4x$, and $4x \times 3x$ crosses (see web Table 5 for the complete data set). We first investigated the two-step pathway of tetraploid formation, which proposes that triploids produced in diploid populations generate tetraploids via backcrossing to diploids, triploid selfing, or crossing among triploids. Tetraploid ($4x$) progeny were observed in four of 18 studies of $3x \times 2x$ crosses, with a mean frequency of 9.8% (range 0–85.7),
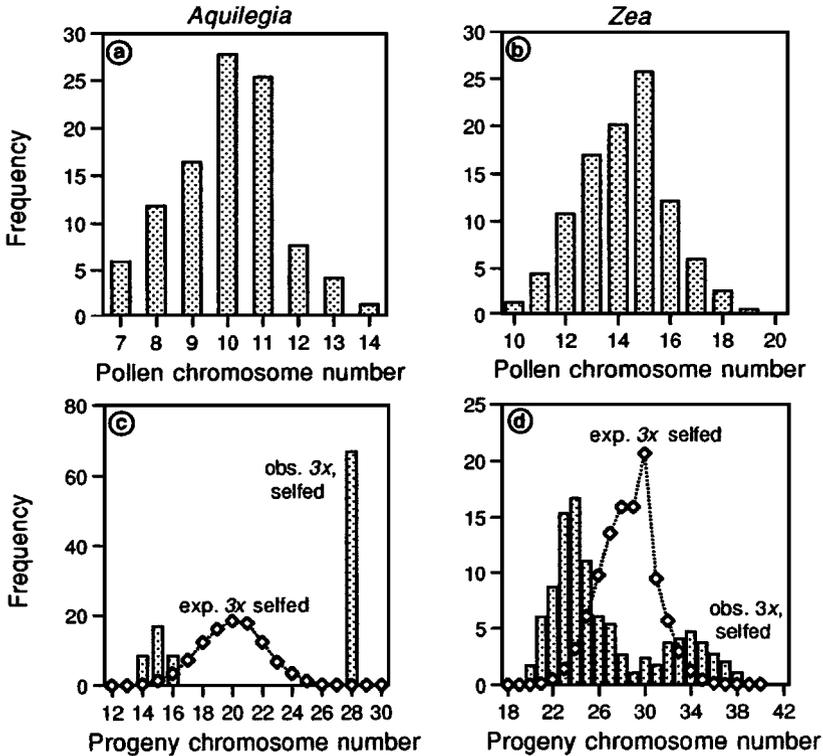
Figure 2 Frequency distribution of pollen chromosome number (*a*, *b*) and observed and expected selfed progeny cytotype (*c*, *d*) in allotriploid *Aquilegia chrysantha* × *flavellata* (*left*) and autotriploid *Zea mays* (*right*). Data from web Tables 3 and 5.

and two of ten studies of $2x \times 3x$ crosses, with a mean of 1.1% (range 0–7.4) (Figure 3*a*, *d*; web Table 5). Tetraploid ($4x$) progeny were observed in one of four studies of $3x \times 3x$ crosses (mean 0.2%, range 0.0–0.8), and in three of eight studies of $3x$ self crosses (mean 13.9%, range 0–66.7) (Figure 3*b*, *e*; web Table 5). Averaged across these four cross types, tetraploids constituted 6.3% of the progeny, suggesting that triploids can contribute to tetraploid formation.

Once some tetraploids have been produced, and mixed cytotype populations have been established, backcrossing between triploids and tetraploids may generate new tetraploids. The likelihood of this type of cross will depend on the relative frequency of the different cytotypes, and the extent of premating isolation between cytotypes. Our survey revealed that tetraploids were common in some $3x \times 4x$ and $4x \times 3x$ crosses, with mean frequencies of 31.6% (range
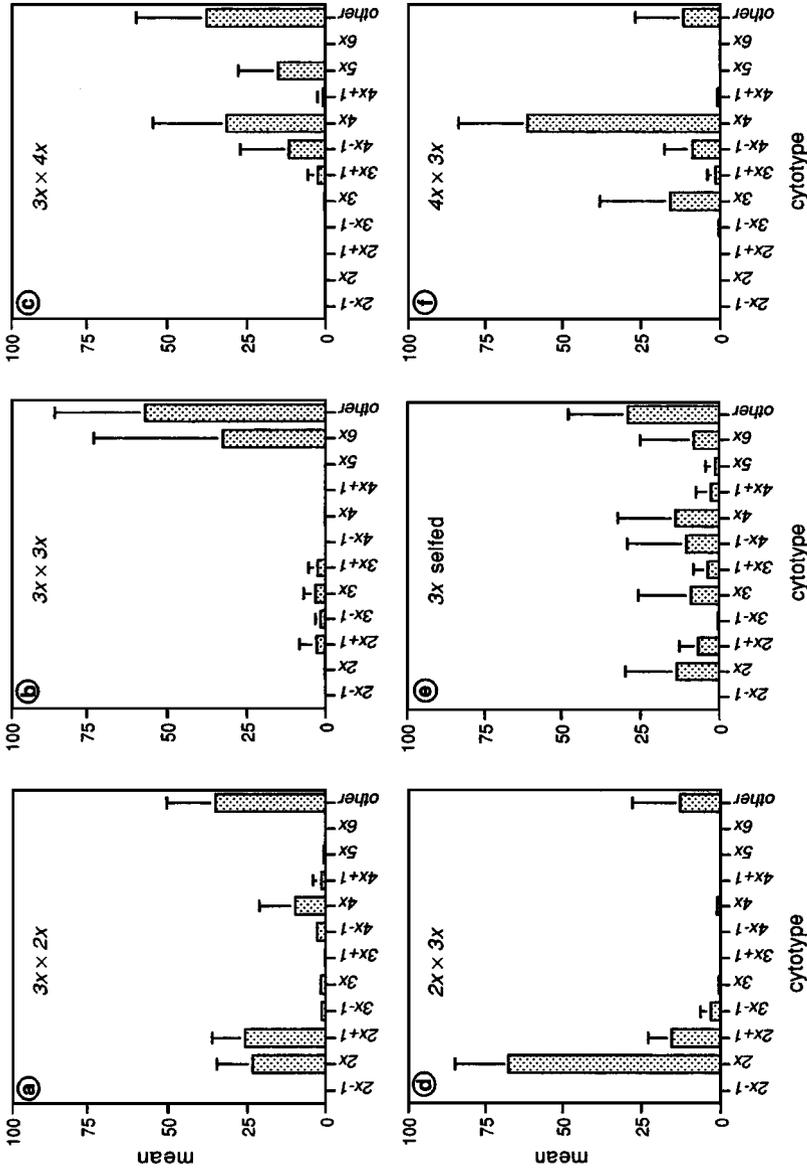
Figure 3 Distribution of progeny cytotype in crosses involving hybrid and nonhybrid triploids. Vertical bars represent two standard errors. Data from web Table 5.

0–100, $n = 10$ studies) and 61.1% (range 35.8–100, $n = 5$) studies, respectively (Figure 3c, f; web Table 5). Thus, tetraploid formation is enhanced under conditions favoring crossing between triploids and tetraploids, as might be expected from phenological differences between diploids and polyploids (99, 104).

These data also allowed us to examine the likelihood of hexaploid formation via a triploid intermediate. Hexaploids were observed in $3x \times 3x$ (mean 32.8%, range 0–81.1) and $3x$ self (mean 8.3%, range 0–66.7) crosses; they are presumably the result of the union of two unreduced ($2n = 3x$) gametes.

The mean frequency of triploids averaged across all cross types was 3.7% (range 0–66.7; web Table 5). Even in crosses between triploids (i.e. $3x \times 3x$ and $3x$ self), which would be expected to generate mostly triploid offspring, the mean frequency of triploids was less than 10% (Figure 3b, e). This suggests that triploids do not perpetuate themselves sexually at a high frequency, and that de novo production via $2n$ gametes in diploid populations, or crossing between tetraploids and diploids, is probably the primary means of triploid production. Tetraploid formation via triploids may thus be facilitated by the perennial habit and vegetative propagation of triploid clones, as has been observed in systems such as *Fritillaria lanceolata* (107) and *Calochortus longebarbatus* (10).

Diploids were recovered in each of the cross types that did not involve tetraploid parents. In crosses among triploids, diploids were observed at frequencies of less than 15% (Figure 3b, e). The frequency was higher in diploid backcrosses, with marked reciprocal differences. In $3x \times 2x$ crosses, the mean frequency of diploids was 23.4% (range 0–78.1), as compared to 67.4% (range 16.4–94.6) in $2x \times 3x$ crosses (Figure 3a, d; web Table 5). It is unclear if this and other apparent reciprocal differences (e.g. tetraploid production in $3x \times 4x$, and $4x \times 3x$ crosses) are related to the production or the viability of the various gamete cytotypes in eggs and pollen.

In general, aneuploids were common in the progeny of triploids. For all cross types combined, the mean frequency of all aneuploid offspring was 50.8%, varying from a mean of 23.2% in $4x \times 3x$ crosses to 64.3% in $3x \times 2x$ crosses (Figure 3; web Table 5). Polyploidy is not thought to evolve as a series of individual chromosome additions involving aneuploids as intermediate steps, because ploidy variation in natural systems generally occurs in complete, or nearly complete, steps (56, 159). Additionally, many aneuploids have low viability and fertility (15, 44, 121). However, the term aneuploid refers to a variety of cytotypes, from those which are very similar to euploids (the "hypo-" and "hyper-euploids," such as $2x + 1$, $4x - 1$), to those with several to many chromosome additions or deletions (e.g. $2x + 4$, $4x - 3$). The meiotic behavior and fertilities of hypo- and hyper-euploid cytotypes are often similar to those of true euploids (15, 121), and there is natural hypo- and hyper-euploid variation in many polyploid species (13, 16, 121). We have therefore distinguished near-

euploids from all other aneuploid classes ("other" category in Figure 3). Hypo-
and hyper-euploids represented, on average, 48.9% of the aneuploids, but the
frequency of such near-euploid cytotypes ranged widely between cross types
(Figure 3; web Table 5). The effective frequency of polyploid formation from
triploids may be significantly influenced by the viability and fertility of hyper-
and hypo-euploid offspring. For example, in $3x$ selfed crosses, if hypo- and
hyper-tetraploids ($4x - 1$, $4x + 1$) are equal in performance to true tetraploids,
the mean production of "tetraploid" cytotypes would increase nearly twofold.
Further research is necessary to determine the roles of different classes of ane-
uploids in polyploid formation. For the reasons discussed above, we consider
$3x - 1$ and $3x + 1$ cytotypes as "triploids" and $4x - 1$ and $4x + 1$ cytotypes as
"tetraploids" in the following sections.

THE FREQUENCY OF SPONTANEOUS POLYPLOIDS

Spontaneous polyploids have been observed in both hybrid and nonhybrid
plant systems. These novel cytotypes are identified on the basis of morpho-
logical characteristics (41, 84, 143, 146), or in the course of cytological surveys
(47, 68, 69, 125). Although the mechanisms responsible for the formation of
these polyploids are often unknown, the frequency of appearance of sponta-
neous polyploids is informative as a direct estimate of the rate of polyploid
formation.

Spontaneous Polyploids Are More Common in Hybrid than in Nonhybrid Systems

To assess the frequency of spontaneous polyploidy, we surveyed the literature
for studies that screened for novel cytotypes in intraspecific crosses, in hybrid
progeny generated by backcrossing, or in F_2 progeny from selfing or interhybrid
crosses (web Table 6). We include hypo-euploid and hyper-euploid cytotypes
(e.g. $2x + 1$, $4x - 1$) in euploid categories. Here we focus on polyploids derived
from diploids, but web Table 6 includes examples of higher-ploidy systems.
The frequency and cytotype of spontaneous polyploids in diploid nonhybrid,
backcross, and F_2 systems differ considerably (Figure 4). New polyploids in
nonhybrid systems are very rare, and are typically triploid. In hybrid systems,
polyploid progeny are often the primary, or only, cytotype produced. The fre-
quency of tetraploids is much higher in progeny derived from F_1 outcrossed
($F_1 \times F_1$) and F_1 self crosses (63%) than from backcross progeny (2%), as
might be expected from the higher frequency of $2n$ gametes observed in hy-
brids than in nonhybrids (Figure 4). However, the frequency of polyploids in
hybrid crosses is higher than would be expected based on the frequency of $2n$
gametes we found previously. For example, on average, a diploid F_1 hybrid
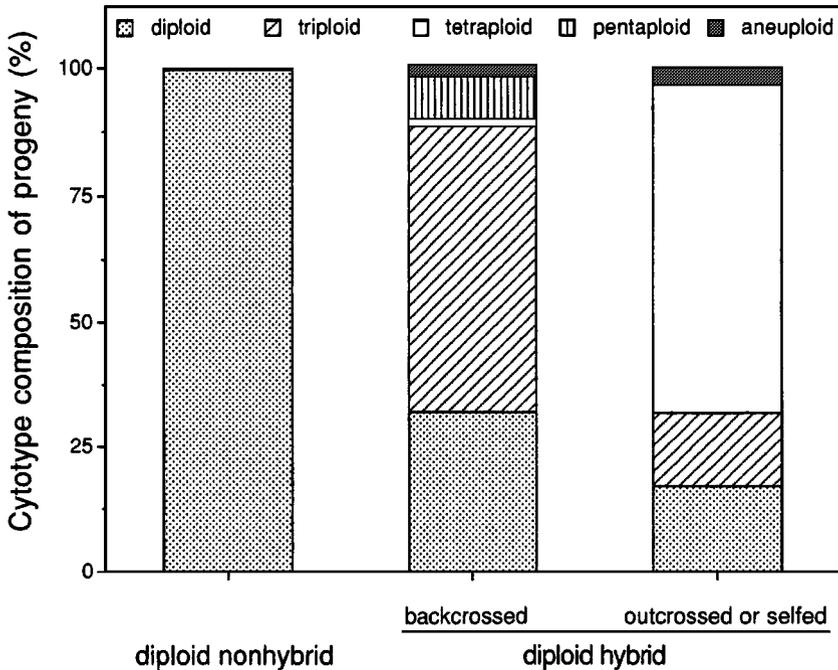
Figure 4 Frequency and cytotype composition of spontaneous polyploids from diploid nonhybrid and diploid hybrid systems. Frequencies of spontaneous polyploids in nonhybrids are 0.203% ($3x$), 0.013% ($4x$), and 0.067% ($5x$). Data from web Table 6.

produces 27.5% $2n$ gametes (web Table 1) and is therefore expected to produce only 7.6% tetraploid offspring on selfing or outcrossing to other F_1s. This difference in tetraploid production (63% vs 7.6%) may represent selection against gametes or progeny that are not polyploid.

Some homoploid interspecific crosses produce polyploids in the F_1 generation, occasionally in unexpectedly high frequencies (53, 60, 79, 93, 152, 163) (web Table 6). For example, crosses between the diploid *Brassica campestris* and *Brassica oleracea* produced four progeny, of which two were triploid and one was tetraploid (169). In Manihot, interspecific crosses produced 1.5% triploids and 2% tetraploids (60). These allopolyploid progeny were the result of $2n$ gametes produced by the nonhybrid parents. Given an average frequency of $2n$ pollen in nonhybrids of 0.05% (web Table 1), the expected frequency of spontaneous triploids and tetraploids is 0.05% and 0.0025%, respectively. The frequency of polyploids in F_1 progeny is commonly higher than these values, suggesting that $2n$ gametes may be at an advantage in some interspecific crosses.

Taken together, these data suggest that the formation of allopolyploids might be more common than that of autopolyploids. However, the rate of allopolyploid formation is a function of both the population-level frequency of hybridization and the rate of polyploid formation in interspecific hybrids. In many of the studies in which spontaneous allopolyploids were observed, it was clear that interspecific hybrids were secured only after considerable effort was made at crossing different species (1, 24, 128). Also, most F_1 hybrids observed to produce polyploids were highly sterile. The mean pollen viability of F_1 hybrids that produced polyploids (Figure 4) was 6.3% ($n = 8$ studies; web Table 7). Seed fertility, though rarely quantified, was often observed to be low (1, 21, 24, 35, 55). For example, Abdel-Hameed & Snow (1) made several hundred crosses between F_1 hybrids of *Clarkia amoena* × *lassenensis* and obtained only a single viable seed, which was tetraploid. After accounting for the ecological isolation that often separates natural species populations (56, 157), the crossing barriers between species, and the low fertility of interspecific hybrids, the overall rate of allopolyploid formation may be much lower than would be expected based only on the observed frequency of spontaneous polyploids from F_1 hybrids.

Allopolyploidy and Disturbed Habitats

The tendency of anthropogenic disturbance to encourage interspecific hybridization by breaking down ecological isolating barriers has long been noted by botanists (5, 56, 157). In the past century, several new allopolyploid species have evolved (106, 133, 145). In each case, the process involved nonnative plant taxa often invading disturbed habitats. For example, the appearance of two new allotetraploid species of *Tragopogon* followed the introduction of the diploid *T. dubius*, *T. porrifolius*, and *T. pratensis* into roadsides and waste areas of eastern Washington state (133). In Britain, the allohexaploid *Senecio cambrensis* was produced by hybridization between the native tetraploid *Senecio vulgaris* and the introduced diploid *Senecio squalidus* (7). Polyploid formation in interspecific hybrids can be essentially automatic (Figure 4), and it is possible that recent allopolyploid evolution is attributable to the high levels of hybridization found in habitats disturbed by human activities.

ESTIMATING THE RATE OF AUTO- AND ALLOPOLYPLOID FORMATION

While there is clear evidence that both auto- and allopolyploids exist in nature, there remains considerable uncertainty regarding the relative frequency of each, and of the factors influencing their abundance. Stebbins (157) and Grant (56) concluded that allopolyploids are much more frequent than autopolyploids (but

see 34), and suggested that this was due in large part to heterosis and home-ostasis conferred by permanent hybridity in allopolyploids, which is lacking in autopolyploids. Another reason to expect a higher frequency of allo- than au-topolyploids is that autopolyploids often show reduced fertility due to meiotic irregularities (34, 159). These potential disadvantages of autopolyploidy are manifest only at the establishment phase, and it is therefore important to con-sider the likelihood of polyploid origins as well, and how the rate of polyploid formation may differ between autopolyploid and allopolyploid pathways. For example, interspecific hybridization is a potentially important rate-limiting step in allopolyploid formation (56). The frequency of $2n$ gametes may determine rates of autopolyploid formation, and a recent simulation model found that the rate of autopolyploid formation also influenced the likelihood of establishment (50).

Evaluating the mechanisms influencing the natural frequency of auto- and allopolyploids requires information on rates of polyploid formation by each pathway. Here we estimate the rate of tetraploid formation via the auto- and allopolyploidy pathways, based on the numerical values of parameters identi-fied in our review of the literature. Our objectives are to (*a*) estimate the total rate of autotetraploid formation, (*b*) determine the relative contribution of the triploid bridge to auto- and allotetraploid formation, (*c*) compare the rates of auto- and allotetraploid formation expected for selfing and outcrossing taxa, and (*d*) compare the rates of tetraploid formation by the two pathways. Be-cause there are few published reports of the frequency of F_1 hybrids in natural populations, this final objective is achieved by estimating the frequency of hy-bridization required to produce equal rates of tetraploid formation via auto- and allopolyploidy.

We believe these analyses are useful because they identify approaches that can be applied to individual natural systems, but we also emphasize several qualifications. The data used to estimate parameters may not be completely in-dicative of natural populations, given that they were taken from a relatively small number of systems, many of which are agricultural or horticultural. Although substantial variation between taxa was observed for many of the relevant parameters, it is likely that our analyses using the mean parameter values provide rough estimates of rates of polyploid formation.

The pathways of tetraploid formation, and the numerical values used to es-timate overall rates, are illustrated in Figure 5. For simplicity, we assume that unreduced gametes are the only cause of polyploid formation. The frequencies of autotriploids and autotetraploids were estimated from $2n$ gamete frequency and triploid block, rather than from estimates of spontaneous autopolyploid formation in diploids, because there are few studies of sufficient sample size to detect spontaneous autotetraploids (web Table 6). The results obtained using

Autotetraploid formation

Triploid-bridge (2 gen.):

	expected freq. 3x			expected freq. 4x from 3x	rate of 4x formation per generation

	freq. 1n	freq. 2n	1- triploid		triploid		freq. 4x (selfing)	
2 X	gametes X	gametes X	block	X	fert.	X	0.274	= 4.98 X 10⁻⁵ (selfing)
	0.99535	0.00465	0.123		0.319			
							freq. 4x (backcrossing) 0.064	= 1.16 X 10⁻⁵ (backcrossing)

expected freq. 4x from 3x

freq. 4x (selfing) 0.274 = 4.98×10^{-5} *(selfing)*

freq. 4x (backcrossing) 0.064 = 1.16×10^{-5} *(backcrossing)*

One-step (1 gen):

expected freq. 4x

freq. 2n gametes 0.00465	X	freq. 2n gametes 0.00465	= 2.16×10^{-5} *(selfing or backcrossing)*

Allotetraploid formation

Triploid-bridge (2 gen.):

hybrid freq. & fitness

freq hybrid. h	X	hybrid fert. 0.0625

exp. freq. 3x

freq. spont. 3x (selfing) 0.14824

freq. spont. 3x (backcrossing) 0.56500

X

triploid fert. 0.319

X

expected freq. 4x from 3x

freq. 4x (selfing) 0.274 = $h\,(4.05 \times 10^{-4})$ *(selfing)*

freq. 4x (backcrossing) 0.064 = $h\,(3.60 \times 10^{-4})$ *(backcrossing)*

One-step (1 gen)

hybrid freq. & fitness

freq hybrid. h	X	hybrid fert. 0.0625

exp. freq. 4x

freq. spont. 4x (selfing) 0.64861 = $h\,(4.05 \times 10^{-2})$ *(selfing)*

freq. spont. 4x (backcrossing) 0.01380 = $h\,(8.63 \times 10^{-4})$ *(backcrossing)*

rate of 4x formation per generation

Figure 5 Estimated rates of auto- and allopolyploid formation via the triploid bridge and one-step pathways. Parameter values for each component were estimated from our review of published studies (see text).

data on spontaneous formation of auto-triploids and tetraploids were similar to those reported here. The frequency of $2n$ gametes for the autopolyploid pathway was estimated by taking the overall mean of the frequencies observed for $2n$ pollen and $2n$ eggs (web Table 1), and triploid block was calculated as the mean of that observed in $2x \times 4x$ and $4x \times 2x$ crosses (web Table 2). The expression for estimating the frequency of autotriploids includes a factor of two to account for allotriploid formation by both unreduced eggs and unreduced pollen (Figure 5). For the allotetraploid pathway, we used the expected frequency of spontaneous allotriploids and allotetraploids appearing in the F_2 progeny produced by selfing or backcrossing F_1 hybrids (web Table 6). This approach was considered more accurate than estimates based on $2n$ gametes. The low

Table 1 Summary of auto- and allotetraploid formation, based on the pathways shown in Figure 5

Type		Selfing	Backcrossing
Autotetraploid	Total rate of formation per generation	7.14×10^{-5}	3.32×10^{-5}
	% of total rate due to 3x bridge	69.7%	34.9%
Allotetraploid	Total rate of formation per generation	$h(4.09 \times 10^{-2})$	$h(1.22 \times 10^{-3})$
	% of total rate due to 3x bridge	1.0%	29.5%
Frequency of interspecific hybridization (h) required for the rate of autotetraploid formation to equal the rate of allotetraploid formation		0.0017	0.0272

viability of "reduced" gametes produced by the irregular meiotic divisions of interspecific hybrids makes the effective frequency of unreduced gametes much higher than would be expected based only on the frequency of gametic non-reduction. We estimated the fertility of F_1 hybrids from experimental studies of allopolyploid formation (web Table 7). For the triploid-bridge in both the auto- and allotetraploid pathways, triploid fertility was estimated as the mean pollen stainability from all studies (web Table 4). To estimate the expected frequency of tetraploids produced by triploids, we used the mean rate of tetraploid formation (defined as $4x - 1$, $4x$ and $4x + 1$) from both auto- and allotriploids resulting from either selfing or backcrossing (web Table 5). The sample sizes used to estimate these parameters are as indicated previously in the text.

The estimated total rate of autotetraploid formation (Table 1) is of the same order (10^{-5}) as estimates of the genic mutation rate obtained from studies in many organisms (57). The rate of tetraploid formation by selfing was greater than that by backcrossing for both autopolyploidy (1.7-fold greater) and allopolyploidy (34-fold greater) (Table 1). The triploid bridge contributes 70% of the total rate of autotetraploid formation in selfing taxa, and 35% of the total in outcrossing taxa (Table 1). In contrast, the triploid bridge contributes only 1% of the total rate of allotetraploid formation in selfing taxa, as compared to 30% in outcrossers.

Most discussions of the role of mating systems in polyploid evolution have focused on establishment, including the contribution of self-pollination to the reproductive isolation of autopolyploids from their diploid progenitors (144, 159), and the selective value of outcrossing in maintaining heterozygosity in autopolyploids (158). We find that self-pollination may play an important role in tetraploid formation, particularly for allopolyploidy. This is consistent with

Grant's (56) conclusion that selfing can facilitate allopolyploid formation, and with the observation by Stebbins (158) that most self-fertilizing polyploids are allopolyploids. In our analysis, the high rate of polyploid formation in selfing allotetraploid systems is attributable to the high frequency of spontaneous allotetraploids in F_1 self crosses (Figure 5). Presumably, this results from self fertilization involving unreduced eggs and unreduced pollen, which may be produced at high frequency in F_1 hybrids. Because of the low frequency of $2n$ gametes in nonhybrid diploid progenitors, the frequency of spontaneous allotetraploids is much lower following backcrossing than following selfing (Figure 5).

Our results indicate that the triploid bridge contributes significantly to autotetraploid formation regardless of the mating system, but is important in allotetraploid formation only in outcrossing taxa. The striking effect of the mating system on the contribution of the triploid bridge to allotetraploid formation is attributed to the higher frequency of unreduced gametes in hybrids than in nonhybrids. Although tetraploid formation is higher in selfing than in outcrossing triploids (Figures 3, 5), this increase is greatly overshadowed by the high frequency of allotetraploids formed by selfing F_1 hybrids (Figures 4, 5). Thus the relative contribution of the triploid bridge is low in selfing allopolyploid systems. In contrast, unless there is a correlation between $2n$ pollen and $2n$ egg, selfing will not increase the frequency of autotetraploid formation via the one-step pathway. Hence, selfing increases the role of the triploid bridge in autopolyploid systems. Overall, the triploid bridge contributes equally to tetraploid formation in outcrossers for both pathways, but has a greater role in selfing auto- than allopolyploid systems.

The estimated frequency of hybridization required for equal rates of tetraploid formation via auto- and allopolyploidy is 0.002 for selfing, and 0.027 for backcrossing (Table 1). The very high frequency of F_1 hybrids required for equivalent rates of auto- and allotetraploid formation in outcrossing taxa seems unlikely in most natural populations. The level of hybridization required for equivalent rates of auto- and allotetraploid formation is much lower in selfing taxa, and this may provide a mechanistic explanation for the observation that self-pollination is the predominant mating system in allopolyploids (158). However, highly selfing taxa will likely have a low frequency of hybridization, so the maximum rate of allotetraploid formation may be expected in taxa with intermediate mating systems.

To estimate the absolute rates of allotetraploid formation requires information on the per generation rate of F_1 hybrid formation in natural plant populations. Surprisingly, despite the widely held view that interspecific hybridization is common in plants (6, 141, but see 48), there are very few estimates of the frequency of F_1 hybrids. The only data for polyploid systems are an estimate

of 0.02% F_1 hybrids in mixed populations of *Senecio vulgaris* and *Senecio squalidus* (109), the progenitors of the allohexaploid *Senecio cambrensis* (145).

Although more data are clearly needed, our results suggest that the frequency of interspecific hybridization required for equivalent rates of auto- and allotetraploid formation is quite high, particularly for outcrossing taxa (Table 1). We conclude that the rate of autopolyploid formation may often be higher than the rate of allopolyploid formation, and that autopolyploidy represents a significant pathway of polyploid formation. The lower relative frequency of autopolyploids reported by many authors (24, 56, 157) may therefore be due more to constraints on the establishment of autopolyploids than to rates of formation (34). Alternatively, the difficulty of detecting autopolyploidy may have biased early estimates of the importance of this mode of formation.

CONCLUSIONS AND FUTURE DIRECTIONS

Although there is general agreement that unreduced gametes are the major mechanism of polyploid formation (19, 24, 63, 165), there is a need for detailed studies examining this and other mechanisms (somatic doubling, endopolyploidy, polyspermy) in natural populations. The cytological and embryological techniques required to examine these phenomena are well developed, but not commonly practiced by many of the plant evolutionary biologists of this generation.

Our review of the literature identified several factors that contribute to the overall rate of polyploid formation. Unreduced gametes are important in both auto- and allopolyploid formation, but their low frequency in nonhybrid systems suggests they have a greater influence on the rate of autopolyploid formation. Our finding of higher crossing success in $4x \times 2x$ than in $2x \times 4x$ crosses suggests that the likelihood of autotriploid formation is higher through $2n$ eggs than through $2n$ pollen. This warrants further study, given the emphasis in the literature on the investigation of $2n$ pollen (11, 24, 111, 132). In hybrid systems, the effective frequency of $2n$ gametes is often very high, and this results in a high rate of polyploid formation in F_1 hybrids. Further research is needed to provide estimates of (*a*) the frequency of $2n$ gametes in hybrid and nonhybrid systems, (*b*) the relative contribution of genetic and environmental factors to gametic nonreduction, and (*c*) the magnitude of spatial and temporal variation in the frequency of $2n$ gametes.

Although theoretical models of polyploid formation and establishment have generally assumed that triploids are either inviable or sterile (50, 144), our results indicate that triploids are often semifertile, and contribute to tetraploid formation. Surprisingly, the mean fertility of F_1 hybrids was lower than that of both auto- and allotriploids, suggesting that F_1 hybrid fertility is perhaps a

greater barrier to polyploid formation. The triploid bridge contributes significantly to the rate of autopolyploid formation regardless of the mating system, and to allopolyploid formation in outcrossing taxa. Our summary of crossing studies revealed that triploid parents rarely produce triploid progeny, which suggests that polyploid formation via the triploid bridge will be favored in perennial taxa. Taken together, these findings demonstrate that future empirical and theoretical work on polyploid evolution should consider the impact of the triploid bridge on polyploid formation and establishment.

Aneuploids are commonly observed in cytological studies of polyploid systems (121, 124), but their role in polyploid formation is not well understood. Issues that need to be addressed include the effects of aneuploidy on viability and fertility, and the consequences of crosses between different euploid and aneuploid cytotypes for polyploid formation.

The estimated rate of autopolyploid formation in both selfing and outcrossing taxa is on the order of the genic mutation rate. New autopolyploids may possess novel physiological, ecological, or phenological characteristics (95) that allow them to colonize a new niche, and are often wholly or partly reproductively isolated from their diploid progenitors. Thus, autopolyploidy may represent a rapid means of adaptation and speciation. The frequency of interspecific hybridization required for equal rates of auto- and allotetraploid formation is so high for outcrossing taxa (2.7%) that this pathway of allopolyploidy is likely only after a breakdown of reproductive isolation, as might be observed in disturbed habitats, or following species introductions. The frequency of interspecific hybridization required for equal rates of auto- and allotetraploid formation (0.2%) is much lower in selfing than in outcrossing taxa, but is still sufficiently high that we suspect the frequency of hybridization is the major rate-limiting step in allopolyploid formation. We suggest that the rate of autopolyploid formation is high and that autopolyploidy is perhaps more common than previously thought, as recent investigations of polyploid taxa have suggested (154, 165).

For both auto- and allopolyploidy, we estimated that the rate of polyploid formation was greater in selfing than in outcrossing taxa. These observations, and the results of theoretical studies showing an advantage to selfing in polyploid establishment (144), demonstrate the importance of the mating system in polyploid evolution. Estimates of the mating system of polyploids and their progenitors are needed to determine the role of self-fertilization in polyploid formation. Phylogenetic studies are of particular interest in this regard to determine if polyploidy is more common in self-compatible lineages.

Despite a long history of cytological and biosystematic research documenting the importance of polyploidy in plant evolution, there are remarkably few empirical studies of polyploids in nature. Comprehensive examination of the pathways, mechanisms, and rates of polyploid formation in natural populations

is a logical next step toward improving our understanding of plant speciation and adaptation. In addition, many questions remain regarding the establishment and persistence of new polyploids. To what extent are new polyploids adapted to novel ecological niches? How much reproductive isolation exists between new polyploids and their progenitors? Is the probability of establishment of a new polyploid related to its mode of origin? Despite nearly a century of research on polyploid evolution, these and other questions remain largely unresolved.

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