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REVIEWS

Review

Evolutionary Dynamics of Unreduced Gametes

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Unreduced gametes, which have the somatic (2n) chromosome number, are an important precursor to polyploid formation and apomixis. The product of irregularities in meiosis, 2n gametes are expected to be rare and deleterious in most natural populations, contrary to their wide taxonomic distribution and the prevalence of polyploidy. To better understand this discrepancy, we review contemporary evidence related to four aspects of 2n gamete dynamics in natural populations: (i) estimates of their frequency; (ii) their environmental and genetic determinants; (iii) adaptive and nonadaptive processes regulating their evolution; and (iv) factors regulating their union and production of polyploids in diploid populations. Aided by high-throughput methods of detection, these foci will advance our understanding of variation in 2n gametes within and among species, and their role in polyploid evolution.

Unreduced Gametes and Formation of Polyploids

The population and evolutionary biology of polyploids have been the focus of much research since the seminal paper on their origins, establishment, and persistence [1]. **Polyploidy** (see Glossary) is common within many taxonomic groups, especially flowering plants and ferns [2–4], and continues to arise repeatedly within many lineages [3,5]. However, questions about its prevalence in natural populations remain unresolved. Much recent work conducted from an ecological perspective has focused on **neopolyploid** establishment, including **frequency-dependent selection** against rare polyploids [6] and the roles of fecundity, **assortative mating**, competition, **mating system**, inbreeding depression and niche differentiation in counteracting this process[1,7–15,87]. By contrast, our understanding of the traits and processes affecting the initial formation of polyploids within diploid populations, and their implications for polyploid evolution generally, is less developed. This gap has been described as one of the underexplored frontiers of polyploid evolution [16].

Although polyploids may form through different mechanisms [17,18], unreduced (2n) gametes involved in sexual reproduction are arguably the primary cause ([1,19–21]; Figure 1, Key Figure). Unreduced gametes contain the somatic (2n) rather than haploid (n) chromosome number and typically arise through irregularities in meiosis [22,23] that interfere with chromosomal segregation or cell division. Despite their role in polyploid formation, our knowledge of the frequency of 2n gametes and the processes that maintain them in natural populations is sparse. Recently, high throughput methods such as **flow cytometry** [24–27] and volumetric analysis [28] have enabled more extensive quantitative assessments of this trait, particularly for male gametes. However, there is no conceptual framework to guide our understanding of the evolutionary dynamics of 2n gametes.

In this review we focus on four questions to evaluate and guide research on the evolution of 2n gametes and their role in polyploid formation in natural populations. (i) How frequently are 2n gametes produced and how are they distributed within and among natural populations?

Trends

Research on the population biology and evolutionary dynamics of polyploidy tends to focus on the establishment phase of polyploid evolution; less research is available on the formation phase.

Recent advances in high-throughput methods (flow cytometry and volumetric measures) enable large-scale sampling and experimental studies of male 2n gametes in natural populations.

Studies of 2n gamete production in natural populations indicate they are widespread; within populations, most individuals have near-zero levels but rare individuals can produce much higher frequencies.

A disparity is observed between 2n gamete frequency estimates in natural populations and rates of neopolyploid production.

Mode of reproduction (including asexual and sexual modes) has been observed to have some bearing on the prevalence of 2n gametes, which may reflect adaptive or nonadaptive evolutionary processes.

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(ii) What are the environmental and genetic determinants of 2n gamete production? (iii) What adaptive and nonadaptive processes maintain 2n gamete production in populations? (iv) What factors determine the fate of 2n gametes and, in particular, whether they contribute to the formation of neopolyploids? Each of these questions should be considered not only within diploid progenitor populations but also their neopolyploid descendants as this will affect diploid–polyploid interactions and the likelihood of neopolyploid establishment. By asking these questions, we identify research opportunities related to the formation and maintenance of variation in 2n gamete production. For simplicity, we discuss these issues in the context of diploid populations giving rise to neotetraploids, but the same principles can be applied, for example, to polyploid populations giving rise to higher polyploids. In addition, we emphasize **autopolyploids** but the general arguments apply to **allopolyploids** as well.

How Frequently Are Unreduced Gametes Produced and How Are They Distributed within and among Natural Populations?

An extensive literature demonstrates that 2n gametes are taxonomically widespread in plants and likely a common mechanism of polyploid formation. An early study examined 17 allopolyploids in 14 genera and found that most arose from 2n gametes produced by diploid hybrids [29]. Later studies documented 106 genera in which 2n gametes likely contributed to polyploid formation [20], and the occurrence of 2n gametes in 20 species across 17 genera [22]. While we know that 2n gametes are commonly present and may contribute to polyploid formation, a significant part of this literature is qualitative in nature, emphasizing only the presence or absence of 2n gametes.

The frequencies of 2n gametes in natural populations are important in determining both the rate of neopolyploid formation and the likelihood of their establishment. Higher frequencies of 2n gametes result in higher rates of neopolyploid formation, regardless of the pathway (Figure 1; [30]). The frequency of 2n gametes will also affect the relative frequencies of different kinds of neopolyploids formed (e.g., 3x vs 4x), thereby influencing the relative rates of neopolyploid formation through **two-step** and **one-step pathways**. In addition, for polyploids to establish neopolyploids must be generated at a rate sufficient for them to persist in the face of the expected **frequency-dependent mating disadvantage** (**minority cytotype exclusion**; [6]). Higher production of 2n gametes by diploids not only generates *de novo* neotetraploids at a faster rate (formation phase), it also increases the probability that neotetraploids will produce tetraploid progeny when they mate with diploids (establishment phase; Figure 1).

Most of what we know about 2n gamete frequencies in plants has come from the crop literature. 2n gamete production is variable among species, cultivars, and even among flowers of an individual [31–33]. At the population level, most individuals typically have near zero frequencies while a few individuals produce high frequencies [17,34,35]. Although this information has value for comparative purposes, it is not obvious that cultivated plant data will be representative of natural populations. The association between polyploidy and cultivated species [36], and past selection for domestication traits [37], suggest that 2n gamete production may have been influenced by artificial selection in at least some cultivated species. Also, crops may differ from natural populations due to the uniformity of growing conditions and frequent reduction in genetic variation. The need for more studies of natural populations was emphasized in the last comprehensive review of the role of 2n gametes in polyploid formation [17]. As a focus for our discussion of what is known for wild populations, we examined the literature in the 20 years since this review [17] and located studies on wild species growing in natural populations, with 2n gamete production under unmanipulated conditions estimated *in situ* (Table 1). We also included two studies in which >10 apparently randomly selected plants were transferred from natural populations to greenhouses [38,39], recognizing that the production of 2n gametes in these cases may differ somewhat from the *in situ* environment.

Glossary

Allopolyploid: polyploids with genome copies derived from more than one species.
Apomeiosis: 2n gametophyte production through incomplete meiotic reduction.
Assortative mating: disproportionately high mating between two individuals of the same type in a heterogenous system (e.g., same-ploidy mating in a mixed ploidy population).
Autopolyploid: polyploids with genome copies derived from a single species.
Clonal reproduction: increase in the number of independent units (ramets) of a given genotype (genet) through vegetative growth and/or production of vegetative propagules.
Flow cytometry: technique in which the DNA contents of nuclei are estimated based on their fluorescence following staining with a DNA selective fluorochrome. Flow cytometry differs from similar methods in that extracted nuclei are suspended in fluid and passed through a laser to excite fluorescence.
Frequency-dependent mating disadvantage: a type of frequency-dependent selection whereby mating success and production of viable offspring by a given phenotype or genotype is positively related to its frequency in a population.
Gametophytic apomixis: a form of seed production without sex in which female apomeiosis is followed by parthenogenesis [71].
Heritability/realized heritability: a measure of the genetic variance relative to total phenotypic variance.
Mating system: description of the sexual system of individuals or populations based on the genetic relationship between mates; falls on a continuum from self-fertilization to cross-fertilization (outcrossing).
Minority cytotype exclusion: the loss of rare cytotypes (often neopolyploids) as a result of frequency-dependent mating disadvantage caused by mating with the majority cytotype.
Neopolyploid (e.g., neotetraploids): a newly formed polyploid. Can refer to a first-generation polyploid with at least one parent of a lower ploidy (*de novo* polyploid) or a member of a polyploid lineage so newly formed it is not yet

In general, *in situ* estimates of 2n gamete production in natural populations are scarce and most estimates are based on few populations (1–4 per species; Table 1). In fact, we found only eight studies meeting our criteria, a point we wish to emphasize because it indicates the slow rate at which this literature is growing. For most species and cytotypes, the recent studies we found agree with the observation that 2n production typically averages from 0.1 to 2.0% in natural populations of nonhybrid species, with most individuals producing zero or low frequencies and a small number of individuals producing substantially more (>10%) [40]. In the broadest study to date, the frequency of 2n gametes was documented in 1696 individuals from 60 populations across 22 species in Brassicaceae; population mean estimates for 19 of these species agreed with the 0.1–2% pattern, although three had some individuals producing >10% 2n gametes (see Table S1 in the online supplemental information, [41]). In the other seven studies in Table 1, most had species with at least some individuals producing high frequencies, but only three species had population means significantly exceeding 2% (female gametes in all cases): *Malus coronaria* (11.6%) [42], *Turnera sidoides* (2.44%) [39], and *Pilosella echioides* (83.8% and 5.77% for 3x and 4x plants, respectively) [38]. Of the six species averaging >2%, one species has significant apomictic seed production (*M. coronaria*, [42]) and two rarely reproduce sexually (*Cardamine* species, [41]) (see "What Evolutionary Forces Maintain Unreduced Gamete Production in Populations?"). The appearance of such exceptions, especially in association

considered established within a population.

Nonadditive sources of genetic variation: contributors to genetic variation whose effects are not simply the sum of effects of different loci or alleles (e.g., dominance and epistasis).

Parthenogenesis: seed formation involving embryo development from an unfertilized egg.

Two-step and one-step pathways: pathways of formation of neopolyploids. Two-step pathways involve two generations and an intermediary cytotype (e.g., triploids in the pathway from diploids to tetraploids); one-step pathways occur in one generation (Figure 1).

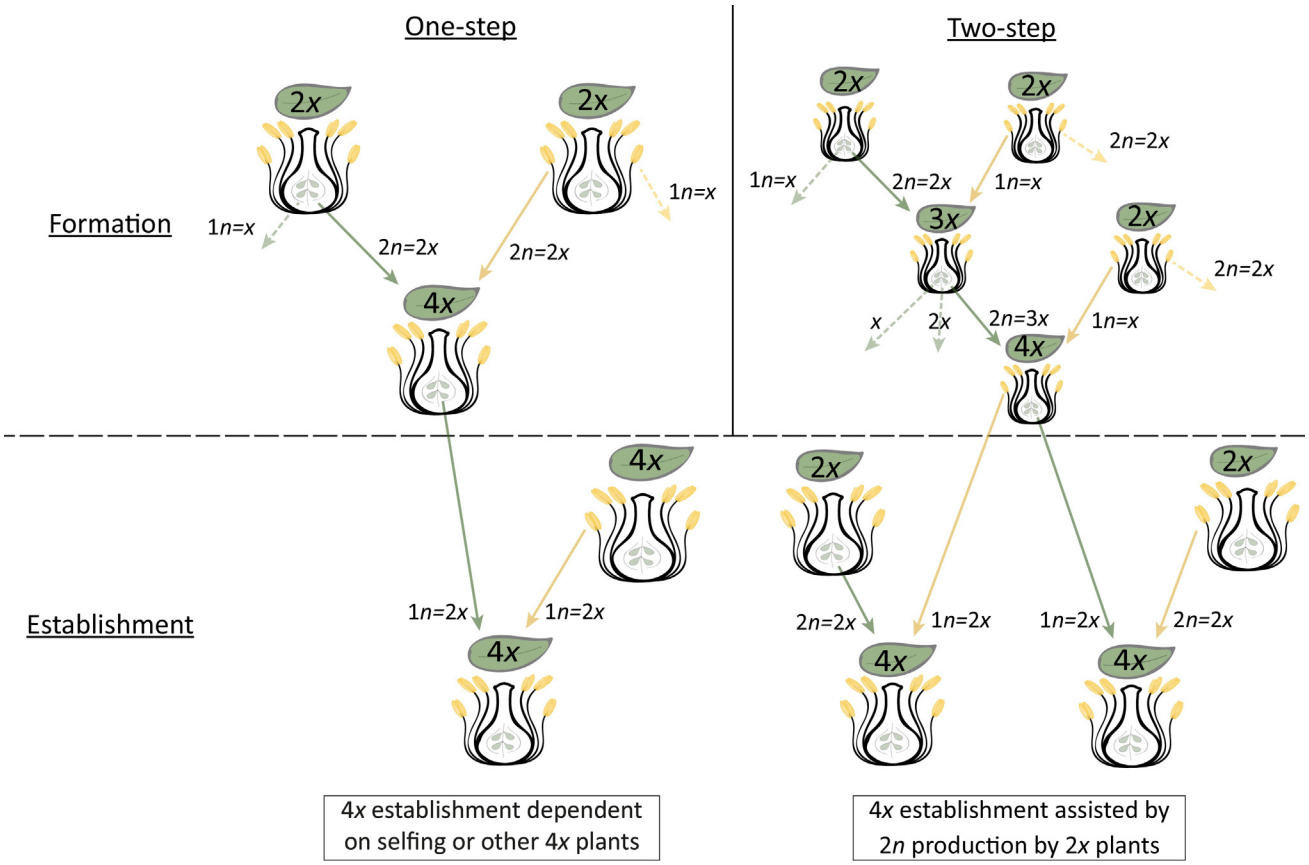

Figure 1. One-Step and Two-Step Pathways to Formation of Neotetraploids. Note that the two-step pathway is possible with only one sex producing 2n gametes (female in the example illustrated but male is also possible), although both sexes may contribute 2n gametes in alternate generations. Yellow arrows refer to male gametes (pollen), green arrows refer to female gametes (ovule). Unbroken arrows and their respective cytotype refer to gametes that contribute to the cross, whereas broken lines are examples of other gamete cytotypes that may be produced by the same individual

Table 1. Attributes of Selected Studies Published since Ramsey and Schemske (1998), in which 2n Gamete Production Was Estimated for Plants from Natural Populations

Study	Species (family)	Populations (plants/ population)	2n % by population or species[s] (mean and range)	2n % by plant (range)	Sex	Method	Cytotype variation?
Bretagnolle, 2001 [43]	*Anthoxanthum alpinum* (2x) (Poaceae)	2 (78–96)	1% (0.8–1.2%)	0–39.5%	Male	Pollen size	Yes (2x)
	A. alpinum (4x)	1 (20)	0% (0%)	0%	Male	Pollen size	Yes (4x)
Kovalsky and Solís Neffa, 2012 [98]	*Turnera sidoides* (Passifloraceae)	1 (50)	1.84%[a,b]	0–12.6%[e]	Male	Pollen size	No (2x)
Kron and Husband, 2009 [42]	*Malus coronaria* (Rosaceae)	1 (12)[c]	11.6%[c]	0–40%[g]	Female	Inter-cytotype crosses	No (4x)
Ramsey, 2007 [40]	*Achillea borealis* (Asteraceae)	2 (100,150)	0.21% (0.08–0.35)	0–15.8%[b]	Male	Pollen size	No (4x)
Sheidai *et al.*, 2009 [99]	5 species[e] (Poaceae)	1–4 (10)	1.35% (0–2.2%)[s]	Not reported	Male	Pollen size	No[e]
Kreiner *et al.*, 2017 [41]	24 species[e] (Brassicaceae)	1–3[d], 7 (2–27)	2.52% (0.4–9.0%)[s]	0–85.6%[f]	Male	Flow cytometry	One species[e]
Kovalsky and Solis Neffa, 2016 [39][g]	*T. sidoides* (Passifloraceae)	1 (12)	2.44%	0–66.7%	Female	Inter-cytotype crosses	No (2x)
Herben *et al.*, 2016 [38][g]	*Pilosella echioides* 2x (Asteraceae)	?[h] (18)	2.1%	Not reported	Male	Inter- & intra-cytotype crosses & flow cytometric seed screening	Yes (2x, 3x, 4x)
		? (18)	0.15%	Not reported	Female		
	P. echioides 3x (Asteraceae)	? (18)	2.2%	Not reported	Male		
		? (18)	83.8%	Not reported	Female		
	P. echioides 4x (Asteraceae)	? (18)	0.08%	Not reported	Male		
		? (18)	5.77%	Not reported	Female		

Populations: Number of populations tested per species (number of plants/population in brackets).
2n % by population or species: mean and range of population means except where indicated as species means (s).
2n % by plant: range of 2n frequencies across all plants in a species.
Sex: Sex of gamete studied.
Method: General method used to assess 2n frequencies.
Cytotype variation?: Whether or not estimates were made for more than one cytotype in a species. Cytotypes listed are all cytotypes for all species in the study.
[a]Average of short-style morph (0.93%) and long-style morph (2.75%).
[b]Includes only 2n gametes, although 4n were also present.
[c]Originally reported 13%; results here are from a reanalysis using only individuals with at least four hybrid seeds tested.
[d]1–3 for 23 of 24 species.
[e]See Table S1 in the online supplemental information for population details.
[f]Across all individuals in all species; see also Table S1 in the online supplemental information.
[g]Plants from natural populations grown in greenhouse for study.
[h]Plants pooled for experiment probably originated from >1 population but this is unclear from the Methods.

with particular reproductive strategies, underlines the need to broaden the taxonomic range of our surveys.

Although three of the eight studies included female gamete estimates, at a species level the estimates are heavily male-biased (32/35 species have male only) (Table 1), likely due to methodological considerations. Studies of male 2n gamete frequencies are predominantly based on measures of pollen size and DNA content (Table 1); equivalently high-throughput measures for female gametes do not exist. For example, DNA content measures of egg nuclei using flow cytometry are complicated (perhaps insurmountably) by the presence of 2n maternal tissue. Direct measures of female 2n gamete production therefore require more challenging

cytological studies of meiosis. All three female gamete estimates (Table 1) are indirect, based on progeny cytotype frequencies from intercytotype crosses or intracytotype crosses combined with flow cytometric seed screening (determination of parental gamete types based on relative ploidies of embryo and endosperm). However, differential fertilization success by 1n and 2n gametes, and differential survival and fertility between the resulting cytotypes, may bias such frequencies away from those actually produced. As a result of these problems, we know less about female than male 2n gamete production in natural populations. This is a particularly important problem if female 2n gamete frequencies are more important for polyploid formation than male [38] and if female 2n gametes differ in frequency from 2n male gametes (as illustrated by [38], Table 1).

Establishment of neopolyploids may be affected not only by 2n gamete production in diploids, but also by 2n gamete production in triploid intermediates in a two-step pathway (Figure 1), and in neopolyploids themselves (e.g., if **gametophytic apomixis** provides reproductive assurance for neopolyploids; see "What Evolutionary Forces Maintain Unreduced Gamete Production in Populations?"). It is therefore important to understand the relationship between the extent of 2n gamete production in diploids and their polyploid descendants. Yet, only three of the eight recent studies of natural populations (Table 1) estimate 2n gamete frequency in more than one cytotype per species. One study includes separate diploid and tetraploid populations [43], and a second includes co-occurring but predominantly asexual 20x, 24x and 30x plants [41]. Only [38] includes potentially sexually interacting cytotypes, although in the artificial context of a greenhouse. It is notable that this last study illustrates the importance of considering all interacting cytotypes, as the male and female estimates differ dramatically both within and between cytotypes. Estimates for diploid, neotriploid, and neotetraploid plants within the same population appear to be otherwise absent in the literature.

The relatively sparse, male-dominated data from natural populations is broadly consistent with studies of cultivated plants in that 2n gametes are relatively infrequent but can vary widely among individuals and species. The low rates observed in most individuals may support the idea that 2n gametes are simply an inevitable byproduct of meiotic errors, but uncommon, high-producing individuals may be important contributors to neopolyploid formation. A few species show higher population mean frequencies, with a possible association with reproductive strategy. Without more intensive surveys of both female and male 2n gametes within and among species and cytotypes under a hypothesis-driven framework, it will be difficult to draw any conclusions about the magnitude of population and species differences, and the genetic and environmental sources of this variation.

What Are the Environmental and Genetic Determinants of 2n Gamete Production?

The potential for evolutionary change in 2n gamete production depends, as with any phenotype, on the presence of heritable genetic variation and its influence relative to environmental and **nonadditive sources of genetic variation** [44,45]. 2n gametes stem from mutations affecting various cytological stages [22,23,46] and have been described for many different species [47–52]. Several premeiotic, meiotic, and postmeiotic pathways that lead to 2n gamete formation have been identified, with key proteins involved in meiotic restitution (DYAD, AtPS1, JAS, TES, and MPK4) and postmeiotic mechanisms (INCENP and RBR) [21]. Some of these genes may be expressed simultaneously (polygenic) [22], which may account for the quantitative variation in 2n gamete frequencies observed in natural populations [41]. However, the extent to which genetic control of 2n gametes within natural populations is governed by few mutations of major effect versus many mutations of small effect is not understood but will affect the tempo and trajectory of adaptive evolution [53,54].

Other important genetic attributes are also worth noting. Some of the genes controlling meiosis have sex-specific expression [55], allowing 2n egg and 2n sperm production to vary independently [56]. This relationship may affect the pathway of polyploid formation, since the one-step pathway requires both 2n male and female gametes (Figure 1). In addition, many 2n gamete mutations are recessive [21,22] but with a high degree of variation in penetrance. These attributes can mask the expression of 2n gametes within heterozygous individuals and thereby slow the response to selection within populations. This effect may be exaggerated in polyploids because of additional gene copies [2].

2n gamete production is also influenced by environmental factors. That a trait with such profound effects on the genomic and genetic attributes of a population can be influenced by environmental variation makes this particularly worthy of investigation. In fact, studies have shown that 2n gamete production may be induced by a variety of factors such as increased herbivory, pathogen attack, nutrition, and water limitation, and notably, fluctuations in temperature [17,57–62]. High environmental lability of 2n gamete production should perhaps not be surprising given the number of cellular mechanisms by which meiosis can be affected; this fact suggests that polyploid formation may be the product of transient environmental fluctuations and occur nonuniformly across the landscape [63]. It also follows that the incidence of polyploidy may be elevated during periods of environmental disruption over short timescales (i.e., within seasons) or long timescales, such as the Cretaceous–Paleogene mass extinction [64] or current climate change.

While genetic and environmental determinants of 2n gamete production have been explored separately, primarily within crop or model systems, their relative impacts and interactions are less often investigated [31,46]. Recent studies with interspecific hybrids involving *Brassica* reporte a genotype × temperature interaction [65] and a wounding × lineage interaction [66] for 2n gamete production. If genotypes vary in lability of 2n gamete production, with some being more sensitive to environmental variation, then we should not expect the likelihood of 2n gamete production and polyploid formation to be uniform across the geographic range of a diploid species.

Estimates of **heritability** for 2n gamete production are needed to predict the short-term response to selection. A small number of estimates exist in agricultural species; for example, the realized heritability of 2n pollen formation was estimated to be 0.5 in *Trifolium pretense* [50]. Another example found realized heritability in *Medicago sativa* of 0.39 and 0.60 for 2n pollen and 2n egg formation, respectively [67]. Both studies suggest a significant portion of variation is heritable although the elevated genetic diversity in natural populations and the heterogeneous environments they inhabit give reason to believe estimates may differ for non-domesticated species. We are not aware of any heritability estimates for 2n gamete production in natural populations nor genetic covariances with other traits, highlighting the need to not only obtain such estimates but also to explore the circumstances in which environmentally induced versus genetic variation may predominate.

What Evolutionary Forces Maintain Unreduced Gamete Production in Populations?

2n gamete production has important reproductive fitness implications and, assuming it is a heritable trait, should be regulated by natural selection. All else being equal, producing 2n gametes should be detrimental to the fitness of diploid individuals. For every 2n gamete a plant produces, it produces two fewer 1n gametes, reducing its potential contribution to 1n + 1n fertilizations and therefore its own fitness. With 2n gametes being rare, most fertilizations involving 2n gametes will result in 3n embryos (e.g., triploids), which typically have low viability and fertility [10,17,68]. Even if 2n gametes go on to produce fertile tetraploids, the frequency of

genes coding for 2n gametes will not increase in the diploid population unless there is a significant transmission of tetraploid genes back into the diploid population. Gene flow between cytotypes is reportedly rare, and when it does occur, is typically from diploids to tetraploids [63,69,70]. Gene flow in the other direction is feasible in some species with fertile triploids capable of producing 1n gametes, but these are rare or nonexistent in many species [10,17]. Even when partially fertile triploids are present, 1n gametes make up only a subset of their gametes (a very small subset, at least in the case of male gametes [17]), and combined with their overall reduced fertility relative to diploids, it is unlikely that their 1n gametes will contribute significantly to the gamete pool of the diploid population. It follows that there should be selection against individuals with 2n gametes, especially high 2n gamete producers; a prediction that has not been directly tested, although it seems to be in agreement with the observation of low mean population levels of production and only infrequent high producers.

If 2n gamete production is maladaptive, how then is it maintained in natural populations? The answer may lie in the population dynamics of deleterious mutations. Specifically, 2n gametes should persist in dynamic equilibrium between the rate of mutation and selective forces acting against them, two parameters about which we know little for 2n gametes. Under this model, one would expect 2n gametes to persist at increased levels when the mutation rates at meiosis are elevated, such as in high ploidy species with genomic instability. Alternatively, they would be elevated when the opportunity for selection against 2n gamete mutations is reduced, as in predominantly asexual species, or when the efficiency of selection is weakened due to low recombination, as in self-fertilizing species. These predictions are largely untested apart from one recent study [41] that found 2n gametes were significantly elevated in asexual compared to sexual species of Brassicaceae. Additional comparative studies across a larger and taxonomically diverse clade are necessary to evaluate the robustness of this hypothesis.

One scenario in which some 2n gamete production may actually be advantageous is in sexually reproducing plants that gain reproductive assurance through some asexual seed production. In the predominant form of gametophytic apomixis, asexual seed production requires both 2n female gametophyte production (**apomeiosis**) and embryo development from an unfertilized egg (**parthenogenesis**) [71], leading to 2n gamete production being favored in the evolution of this trait [72]. While selection for or against 2n egg production may vary spatially and temporally, if it plays a strong enough role in providing reproductive assurance in an otherwise sexually reproducing species, higher frequencies may be maintained than in other systems. An interesting implication of this idea is that the fitness benefits may only apply to 2n egg production—2n sperm production would still have consistent fitness costs. This would provide a selective basis for decoupling male and female 2n gamete production, favoring high female and low male 2n gamete frequencies. One consequence might be increased prevalence of the two-step pathway of neopolyploid production involving 1n sperm uniting with 2n eggs in diploids (step one) and then triploids (step two). 2n egg production in triploids could be inherited from the diploid mother [73] and/or selectively favored for the same reason as in diploids.

It has been proposed that induction of 2n gametes in stressful environments may be selected to enhance polyploid production in habitats where they are most expected to thrive [63,64]. The argument is that, assuming polyploids will have higher relative fitness than diploids in those particular environments, 2n gametes may be advantageous in an across-lineage sense, in that their production gives rise to a new, successful, polyploid lineage, which carries the 2n gamete-promoting gene [74] (although the impact of polyploidization on species diversification rates is debated [75–77]). While this idea could be useful for explaining why 2n gamete production may be a common feature of newly formed polyploid lineages, it cannot explain the maintenance of and variation in 2n gamete production within populations and species. Within either the diploid progenitor or polyploid derivative, plants producing 2n gametes will still experience a fitness

disadvantage in most cases. Furthermore, there is no widely applicable mechanism for increasing the frequency of an inducible 2n gamete gene in the parental (diploid) population aside from gene flow from the higher ploidy back into the parental population, which is likely low or nonexistent in most cases (as discussed above). Thus, the role of 2n gametes in participating in inducible neopolyploid formation in select environments does not address the key evolutionary issue of how 2n gametes are maintained within microevolutionary time scales.

What Factors Determine the Fate of Unreduced Gametes in Populations and Their Contribution to Neopolyploid Formation?

The contribution of 2n gametes to polyploid formation depends not only on their mean frequency in a population, but also on factors affecting their relative success during mating and recruitment. The fates of 2n gametes can be partitioned into three stages: (i) pollen dispersal and deposition (pollination); (ii) gamete competition for fertilization opportunities (postpollination, prefertilization); and (iii) survival and establishment of seeds (postfertilization) [78]. The combined success of 2n gametes across these stages determines whether they will form polyploid offspring and thus may account for heterogeneity in the incidence of polyploidy among plant groups [79]. Research on stage iii, the relative fitness of progeny formed by one or two 2n gametes, is extensive and convincingly shows that n + 2n offspring (e.g., triploids) have low if not lethal viability (triploid block) and low fertility compared to n + n or 2n + 2n offspring [17,68]. The magnitude of this effect varies among taxa and depends on whether the egg or sperm is 2n [17,80,81] as well as the ploidy of the endosperm [82]. In most cases, the result is selection against odd-ploidy cytotypes and the two-step pathway of polyploid production in many species (see [10]). Rather than revisiting this literature, below, we focus our attention on stages i and ii.

Stages i and ii relate to the way in which the mobile and more numerous male gametes are distributed and how they compete for fertilization opportunities. Pollen dispersal patterns can affect polyploid formation by controlling the union of 2n gametes. For example, self-pollination can enhance one-step polyploid formation by increasing the rate of assortative mating with respect to 2n gametes [83]. That is, if 2n gamete frequency is variable among individuals and correlated between pollen and ovules, then self-pollination will increase the likelihood that 2n gametes will unite (i.e., 2n + 2n). Random cross-pollination, with the same population frequency of 2n gametes, will produce a higher proportion of polyploids overall but most will be of the n + 2n type (e.g., triploids) which, as stated, usually have lower fitness [17,68]. Thus for 2x/4x species with few or no fertile triploids, self-pollination is expected to yield more spontaneous neotetraploids than cross-pollination. If triploids are viable and partially fertile, neotetraploid production via the two-step pathway may also be elevated by selfing compared to outcrossing, since it ensures the 1x, 2x, and 3x pollen in triploid intermediaries combine. This is true for both auto- and allopolyploid formation, especially the latter [17], as long as 2n gamete production covaries in pollen and ovules, an assumption that has not been fully tested.

Population substructure can achieve a similar effect to selfing by enhancing pollen dispersal between genetically similar individuals. Substructure can arise through divergent selection or restricted dispersal of seeds [84,85]. When substructure is present, local dispersal of gametes can increase the chance of 2n + 2n unions compared to random pollination. As with selfing, this outcome requires that male and female 2n gamete frequencies are correlated among individuals, that plants are not self-incompatible, and that pollen dispersal is spatially restricted, which is widely observed in plants [86]. Regardless of mechanism, assortative pollination and correlated frequencies of male and female 2n gametes lead to the prediction that species with predominant self-fertilization or population substructure are more likely to form spontaneous neopolyploids. This process may help to explain associations between the incidence of polyploidy and both self-fertilization [9,87,88] and **clonal reproduction** [63,89,90].

The contribution of 2n gametes to polyploid formation also depends on their ability to compete for fertilization opportunities. In flowering plants, this manifests itself through competition among pollen nuclei for access to ovules during pollen tube growth in the style (see [91] for differential ovule development). There are few experimental comparisons of siring success between 2n and 1n pollen. In the perennial plant, fireweed, 2n pollen grains have similar or greater siring success than 1n pollen when placed in pollen mixtures on diploid and tetraploid flowers, respectively [92,93]. By contrast, 1x and 3x gametes from diploid and hexaploid species of *Betula*, respectively, sire most of the eggs within their own cytotype [94]. Siring success of 2n pollen may differ from 1n pollen for several reasons. 2n gametes tend to be larger and may retain more limiting resources than 1n pollen, allowing them to grow farther or faster in the style [95]. In addition, 2n gametes contain multiple copies of each gene (two copies in diploids) and, therefore, may benefit from masking of deleterious recessive mutations affecting pollen tube growth [2]. Both of these mechanisms enhance the siring ability of 2n pollen relative to 1n pollen. To date, however, only one study has examined the role of masking in 2n pollen [96]. No studies have isolated the consequences of pollen or ovule size to siring success in 2n gametes, although evidence for a role of size is mixed in diploid plants [95,97]. Counteracting these potential advantages is the fact that 2n gametes are prone to uneven chromosome complements (aneuploidy) and higher sterility as a result of meiotic dysfunction. The relative importance of these factors in determining access to fertilization by 2n gametes is unclear.

Taken together, the fate of 2n pollen is the product of three independent stages: pollination, postpollination/prefertilization, and postfertilization survival. As a result of selection at each of these potential barriers, polyploid formation via one-step or two-step pathways is expected to deviate from that predicted by the frequencies of 2n gametes alone. This prediction is corroborated by [43], who found that frequency of triploids/tetraploids in open-pollinated seeds was only one tenth that expected based on the frequency of 2n pollen (estimated by size). The magnitude of this discrepancy in most systems remains unknown.

Concluding Remarks

2n gametes result from meiotic irregularities that interfere with chromosomal segregation or cell division. Although the product of meiotic errors, 2n gametes are precursors to important evolutionary innovations such as polyploid evolution and apomixis. Due to their environmental lability, they may also be valuable indicators of ecological change. As research into the frequencies of 2n gametes in natural populations increases, it will be important to motivate and guide this growth using an evolutionary framework. We explored four key areas pertaining to the processes maintaining seemingly deleterious traits in natural populations across many species (see Outstanding Questions). Combined with high-throughput methods of detecting 2n gametes, these research directions are poised to change the way we think about this important stage of polyploid evolution.

Supplemental Information

Supplemental information associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tig.2017.06.009.

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Outstanding Questions

How are 2n gamete frequencies distributed in natural populations of angiosperms? What are their phylogenetic, genetic, and environmental sources of variation? Are different patterns associated with different environments, reproductive modes, life histories, etc.?

How do male and female 2n frequencies differ? Do they tend to be correlated (similar within individuals)? Do female 2n gametes play a greater role in neopolyploid formation than male? How can we improve our knowledge of female 2n frequencies?

Do neopolyploids tend to show similar 2n gamete production phenotypes as their parents? Does this typically reflect the parental population mean? Does any such pattern apply only to early generation neopolyploids?

What is the genetic architecture of 2n gamete production across species? How heritable (evolvable) is this trait? How important are genotype–environment interactions? Is it possible to make broad generalizations about genetic architecture that commonly apply in natural populations?

What is the relationship between 2n gamete production and fitness? Are fitness advantages required to explain 2n gamete frequencies in natural populations? If so, what are these advantages? Do facultative apomicts represent a special case? How does strength of selection for or against 2n gametes vary among reproductive modes (including sexual/facultatively asexual, mating systems)?

Why do we see a disparity between estimates of 2n production and rates of neopolyploid formation? What role does assortative mating play? To what extent can these disparities be explained by (i) differential fitness of gametes (1n vs 2n) and embryos (2n v2 3n vs 4n) and (ii) problems with 2n gamete estimates (e.g., lack of female estimates; methods that overestimate 2n)?

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