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# Polyploid organisms

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Polyploids are organisms with three or more complete chromosome sets. Polyploidization is widespread in plants and animals, and is an important mechanism of speciation. Genome sequencing and related molecular systematics and bioinformatics studies on plants and animals in recent years support the view that species have been shaped by whole genome duplication during evolution. The stability of polyploids depends on rapid genome recombination and changes in gene expression after formation. The formation of polyploids and subsequent diploidization are important aspects in long-term evolution. Polyploids can be formed in various ways. Among them, hybrid organisms formed by distant hybridization could produce unreduced gametes and thus generate offspring with doubled chromosomes, which is a fast, efficient method of polyploidization. The formation of fertile polyploids not only promoted the interflow of genetic materials among species and enriched the species diversity, but also laid the foundation for polyploidy breeding. The study of polyploids has both important theoretical significance and valuable applications. The production and application of polyploidy breeding have brought remarkable economic and social benefits.

**polyploid, whole genome duplication, diploidization, distant hybridization, polyploidy breeding**

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Polyploids are organisms with three or more complete chromosome sets. Some studies have shown that all of the angiosperms are paleopolyploids, and have experienced one or several rounds of polyploidy during their evolution [1]. According to the indexes of genome size and isozyme complexity, Ohno put forward the hypothesis of genome duplication in 1970, and thought that two rounds (2R) of whole genome duplication (WGD) occurred in early vertebrate evolution [2]. The first duplication happened in the evolution process from Cephalochordates to jawless vertebrates; and the second one was after the divergent evolution of hagfish and lampreys [3]. Studies of Hox genes and Hox gene clusters strongly support this hypothesis [4]: researchers found a 1∶2 or 1∶4 distribution of Hox clusters in various invertebrates and vertebrates [5,6]. In recent years, data based on vertebrate genome sequencing, phylogenetic trees, and genomic map position have provided further evidences to support the genome duplication hypothesis. In addition, the ray-finned fish experienced a third genome duplication: the fish-specific genome duplication (FSGD or 3R), after their separation from the tetrapods [7–9]. The polyploids formed through genome duplication would display changes in their genome structure, gene regulation, and expression, and would subsequently become new diploids or paleopolyploids through diploidization and differentiation [10–12]. The new diploids and paleopolyploids could then form new polyploids, further enriching the species resources. A systematic discussion and summary of the types of plant and animal polyploids, and the genesis and genetic influence of polyploidy, have both important theoretical value and practical applications for genetic breeding. In this paper, we present a systematic exposition of the research progress of

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polyploid organisms, particularly polyploid fish.

## 1 Polyploid plants

### 1.1 The frequency of polyploidy in various groups of plants

Polyploidization is widespread in plants. Polyploids in bryophytes comprise about 53% of the total species; *Plagiomnium medium* is an allotetraploid, for example [13]. The frequency of polyploidy in pteridophytes could be as high as 95% [14]. About 38% of gymnosperms are polyploids. California redwood (*Sequoia sempervirens*, 2*n*=6X=66) is the only natural hexaploid conifer in existence, and is also one of the fastest-growing species in the world [15,16]. Through one round of polyploidization and hybridization, their ancestors formed AAB-type plants, and then doubled again, generating the existing hexaploid redwood. In addition, there are triploid or tetraploid species in *Cryptomeria*, cypress, red pine, spruce, and larch.

Studies on angiosperm polyploids are extensive, and have been going on for more than a century. Early in the 20th century, *gigas*, a mutant in Lamarckian primrose (*Oenothera lamarckiana*), was found to be a kind of tetraploid [17]. It has proved difficult to determine the actual frequency of polyploids in various plant lineages, despite numerous attempts. Grant's estimate was based on the assumption that the ancestral number of chromosomes in angiosperms was 7–9 and that any flowering plant with $n \geqslant 14$ chromosomes had undergone polyploidization at some point during angiosperm evolution [14]. Grant postulated that 47% of all flowering plants were of polyploid origin; he proposed that 58% of monocots and 43% of dicots were polyploids. Goldblatt thought that taxa with chromosome numbers above *n*=9 or 10 would have undergone polyploidization in their evolutionary history; he calculated that 70%–80% of monocots were of polyploidy origin [18]. Using a similar approach in dicots, Lewis estimated that 70%–80% among them were polyploids [19]. Masterson estimated that 70% of all angiosperms had experienced one or more episodes of polyploidization in their ancestry [20]. Recent genomic analyses indicate that an early polyploidization event may predate the radiation of flowering plants, suggesting that 100% of angiosperms are paleopolyploids [1,21].

### 1.2 Molecular biological research on polyploidy plants

Comparisons of diversification rates suggest that genome doubling may have led to a dramatic increase in species richness in several angiosperm lineages, including Poaceae, Solanaceae, Fabaceae, and Brassicaceae [22]. Studies on MADS-box genes duplication and analyses of protein-protein interaction networks among MADS-box proteins [23], also support genome doubling early in angiosperm history. In the Floral Genome Project, the approach of the frequency distribution of per-site synonymous divergence levels (*Ks*) for pairs of duplicate genes was applied to expressed sequence tags (ESTs) from a suite of basal angiosperms [24], and evidence was found for episodes of ancient genome-wide duplication in *Nuphar advena* (Nymphaeaceae), *Persea americana* (Lauraceae), *Liriodendron tulipifera* (Magnoliaceae) and *Saruma henryi* (Aristolochiaceae). Moreover, Cui *et al*. detected independent genome duplications in California poppy (*Eschscholzia californica*; Papaveraceae) and sweet flag (*Acorus americanus*; Acoraceae), both of which were distinct from the duplications documented for core eudicots and Poaceae [25]. According to the current study, the formation of diploid, triploid, tetraploid, and hexaploid is suggested in the long-term natural variability of sweet flag development [26].

Whole-genome sequencing is conducive to the complete and systematic study of the polyploidy phenomenon. The complete sequencing of *Arabidopsis thaliana* revealed numerous duplicate genes and suggested two or three rounds of genome-wide duplication [27]. Additional evidence indicated that before the two rounds of whole genome replication, *Arabidopsis* also experienced an earlier whole genome triplication (WGT) process [28]. This mode was also true for the evolutionary process of soybean (*Glycine max*), which went through three rounds of polyploidization, comprising one round of hexaploidy and two rounds of tetraploidy [28]. Based on analyses of the complete genome sequence of grape (*Vitis vinifera*), Jaillon *et al*. revealed that the common ancestor of grape, poplar (*Populus* spp.), and *Arabidopsis* was an ancient hexaploid that possibly arose after the divergence of the monocot and eudicot lineages [29]. Evidence in the papaya (*Carica papaya*) genome also supported the genome triplication in this species [30]. Severin hypothesized that when *Arabidopsis*, grape, soybean, poplar, and papaya were included in this common model, it indicated a shared ancestor containing seven chromosomes [28].

### 1.3 Familiar types of polyploidy plants

Some familiar crop species, such as sugar cane (*Saccharum*), coffee (*Coffea Arabica*), cotton (*Gossypium gossypioides*, 2*n*=4X=52), and tobacco (*Nicotiana tabacum*, 2*n*=4X=48), are polyploids [31]. There are several polyploids in the genus *Triticum*: both emmer wheat (*Triticum dicoccum*, 2*n*=4X=28) and *Triticum dicoccoides* (2*n*=4X=28) are tetraploid, while common wheat (*Triticum aestivum*, 2*n*=6X=42) is allohexaploid. Banana (*Musa nana*, 2*n*=3X=33), daffodil (*Narcissus tazella*, 2*n*=3X=30), and daylily (*Hemerocallis fulva*, 2*n*=3X=33) are all autotriploids [32]. Two kinds of potatoes, *Solanum stoloniferum* (2*n*=4X=48) and *Solanum demissum* (2*n*=6X=72) are tetraploid and hexaploid, respectively [33]. Additionally, some vegetables, such as taro (*Colocasia esculenta*, 2*n*=3X=42), chive (*Allium*

*schoenoprasum*, 2*n*=4X=32), Chinese yam (*Disoscorea batatas*, 2*n*=4X=40), and sword bean (*Canavalia gladiata* (Jacq) DC., 2*n*=4X=44), are natural polyploids [34].

Fedorov added up chromosome numbers of 93 plants in chrysanthemum, and found 56 kinds of polyploids (including intraspecific polyploids), accounting for 60% [35]. Thus, polyploidization is apparently an important evolutionary path in this genus. Among wild chrysanthemum for example, there are diploids, allotetraploids, hexaploids, and decaploids [36]. In the wild primrose, some polyploids have been found, such as tetraploid *Primula malacoides* (2*n*=4X=36), hexaploid *P. incana* (2*n*=6X=54) and *P. scotica* (2*n*=6X=54); octoploids and decaploids are also reported [37].

## 2 Polyploid animals

There are growing evidences that extensive polyploidy also existing in animals. Nearly 200 independent examples of polyploids have been reported in insects and vertebrates, in addition to the many more cases known among other invertebrate groups [1,38].

### 2.1 The polyploids in invertebrates

In invertebrates, the polyploids in Turbellaria and Oligochaeta are usually hermaphrodites. *Pontoporeia affinis*, the glacier residue of Crustaceans, is a sexual polyploid. While *Artemia salina* of Branchiopoda and *Trichonicus* sp. of Isopod are asexual [39]. There are different subspecies of diploids (2*n*=2) and tetraploids (2*n*=4X=4) in *Parascaris equorum* of Nematomorpha [32]. In Mollusca, mainly female hexaploids (2*n*=6X=90–94) in Melaniidae have been reported. Ancylidae also show polyploidy. For example, Ethiopic *Ancylus* sp. (2*n*=4X=60) is tetraploid, and England *Ancylus fluviatilis* (2*n*=8X=120) is octoploid [40].

There are estimated to be 2.5–3 million types of insects, but less than 100 are known to be polypoids, distributed in every major order [41]. For example, tetraploid *Saga pedo* is found in Tettigoniidae, triploid *Psychoda parthenogenetica* in Psychodidae, and tetraploid *Diprion* in Tenthredinidae. Polyploids in Curculionidae are the most abundant, comprising 38 triploid, 17 tetraploid, five pentaploid and two hexaploid parthenogenetic subspecies or species. The polyploid insects survive by parthenogenesis. Most of them cannot fly, and they are small. Their living environment is relatively stable; thus, they can live a longer life [42].

### 2.2 Polyploid vertebrates

Humans and other land vertebrates probably share a 360–450 million-year-old fish ancestor, and the common ancestor contains a genome with 12 chromosomes [9,43]. Early in vertebrate evolution, through two rounds of whole genome duplication and various kinds of chromosome rearrangement, the ancestor gave rise to the species consisting of the current 20–30 haploid set [44]. The species types of ray-finned fish correspond to more than half of all vertebrate species, and the group has gone through another round of fish-specific genome duplication [7–9]. Genome duplication results in better environmental adaptation, and plays an important role to the evolution of fishes and land vertebrates [2].

As the lower vertebrates, some polyploid fishes thrive by way of parthenogenesis, which are often related to hybridization events [45]. Gibel carp (*Carassius auratus gibelio*) can reproduce by gynogenesis, and two kinds of triploids, with 156 or 162 chromosomes, were found by karyotype analysis [46]. A related study considers that the gibel carp are entering into an evolutionary trajectory of diploidization. The coexistence of different ploidy populations (diploid, triploid and tetraploid) among crucian carp (*Carassius auratus*) of the Dongting water system was found [47]. These polyploids might be generated by hybridization. Studies have shown that the triploid containing microchromosomes can produce offspring by gynogenesis or sexual reproduction, while the tetraploid can only generate all-female offspring by gynogenesis. The reproductive mode of gynogenesis also exists in Dianchi High-back crucian carp in Yunnan, China [48]. According to the view of whole genome duplication, these types of triploid should actually be regarded as hexaploid. In addition, some unisexual triploidy species in Cyprinodontiformes are mostly of hybrid origin. For example, the Amazon molly (*Poecilia formosa*) is a hybrid between *P. latipinna* and *P. mexicana*. When its gynogenesis diploids backcross with *P. mexicana*, gynogenesis triploids could be formed [48,49].

In some fish families, all the members studied originate from a tetraploid [50], such as Salmonidae, Coregonidae, Catostomidae, and Thymallidae. Additionally, triploidy or tetraploidy origin exists in Siluridae, Callichthyidae, and Loricaridae.

The formation of certain fish groups in Cyprinidae is related to polyploidy [50]. Many species belonging to *Cyprinus*, *Carassius*, or *Barbus* are obvious tetraploids (2*n*=4X=100). *Schizothorax* sp., *S. grahami,* and *S. daliensis* in Schizothoracinae are all hexaploids. In Cobitidae, *Misgurnus fossilts* is a tetraploid. Coexistence of natural polyploids and diploids was also found in *M. anguillicaudatus* [51], *Cobitis taenia*, and *C. elongatoides* [52,53].

In amphibians and reptiles, the number of polyploid species and populations is increasing. Almost all the polyploids are related to diploid relatives, and many of them have morphologically very similar diploid isomorphic species, but there are no obvious polyploid families or genera [39]. In Anuran, *Ceratophrys dorsata* (2*n*=8X=104) from Brazil and *C. arnata* from Argentina are octoploids. *Xenopus ruwenzoriensis* (2*n*=6X=108) in Uganda is hexaploid. African clawed frog (*Xenopus vastitus*, 2*n*=4X=72) and America

rain frog (*Hyla versicolor*, 2*n*=4X=48) are tetraploid. In addition, *Bufovtridis* in Xinjiang is a tetraploid (2*n*=4X=44). There are almost no triploids among Anuran, with the exception of North America's *Rana esculenta* (2*n*=3X=39). In *Ambystoma* of Caudata, *A. platineum*, *A. tremblayi*, and *A. texanumxlaterale* are all triploid gynogenesis types (2*n*=3X=42). *Siren lacertima* (2*n*=4X=52) and *Pseudobrachis striatus* (2*n*=4X=64) are sexual tetraploids [54].

All polyploids in Reptiles are triploids, and produce parthenogenetic progenies [55]. *Cnemidophorus neomexicanus*, *C. uniparenus*, and *C. exsanguis* (2*n*=3X=69) of Teiidae, are mainly distributed in Central America. In Gekkonidae, *Gehyra variegate ogasauarisimae* (2*n*=3X=63), *Hemidodactylus garnotii* (2*n*=3X=70), and *Heteronotia binoei* (2*n*=3X=63), come from Japan, Australia, or other places. *Leiolepis belliana* (2*n*=3X=54) and *L. tripoida* (2*n*=3X=54) in Agamidae are widespread in Malaysia.

Higher vertebrates do not tolerate polyploidy [11]. Some scholars believe that the golden hamster (*Mesocicetus auratus*) is a polyploid, but it has been proved to be a pseudo-tetraploid [56]. Among chicken embryos, only 0.9% are triploid or tetraploid. In mammals and birds, ploidy changes are typically fatal, with polyploids dying early during development [1].

## 3 The occurrence of polyploids

### 3.1 Mechanisms of polyploid formation

The formation of polyploid organisms has mainly occurred via three routes: chromosome doubling, gametic nonreduction, and polyspermy [31,57].

Chromosome doubling in plants and animals is related to a failure of cell division following mitotic doubling. It may occur in the zygote, young embryo, or meristem of a plant, and will ultimately lead to the production of polyploidy tissues and the generation of minority polyploids [10,57]. The classic case is *Primula kewensis* [58], a tetraploid in *Primula*. It originated from fertile tetraploid shoots whose chromosomes doubled on sterile $F_1$ plants of *P. floribunda*×*P. verticillata*. The naturally occurring tetraploid among Lamarckian primrose was formed through zygote chromosome doubling [17]. On the other hand, in animals, when the distant hybridization of red crucian carp (*Carassius auratus* red var., 2*n*=100) and blunt snout bream (*Megalobrama amblycephala*, 2*n*=48) occurred, in the first generation, the first cleavage of some fertilized eggs was inhibited, causing the chromosome number to double, resulting in tetraploids [59,60].

The union of two unreduced gametes, or of reduced and unreduced gametes, is a major mechanism of polyploidization in plants [31]. For example, triploids and pentaploids occured in the progenies of the open-pollinated diploid *Crepis capillaris*, and these appeared to have been produced by the fusion of reduced (*n*) and unreduced (2*n* and 4*n*) gametes [57]. The hybridization of *Raphanus sativa* (2*n*=18) and *Brassica oleracea* (2*n*=18) could produce allotetraploid offspring (*Raphanobrassica*, 2*n*=4X=36) in the $F_2$, because a small number of germ cells in the $F_1$ generated unreduced gametes [61]. The autotetraploid *Dactylis glomerata* and allotetraploid *Tragopogon* were also produced in the same way [10].

In animals, the descendants of distant hybridization possess a reproductive characteristic to produce unreduced gametes. In the distant hybridization of red crucian carp (2*n*=100)×common carp (*Cyprinus carpio* L., 2*n*=100), some individuals produced unreduced gametes, then the unreduced eggs were fertilized with unreduced diploid spermatozoa to produce bisexual fertile allotetraploid (4*n*=200, or 2*n*=4X=200) in the $F_3$ [62]. The diploid gynogenetic clonal line, which was formed by gynogenesis on diploid eggs of the allotetraploid, has the ability to stably produce unreduced diploid eggs [63]. The mechanism of the formation of the unreduced gametes is related to the behavior of germ cells before meiosis [60,64], which may have involved premeiotic endoreduplication or endomitosis, or fusion of oogonia or spermatogonia. Normal meiosis is carried out after chromosome doubling of germ cells, thus results in unreduced gametes.

The generation of unreduced gametes is also related to meiotic abnormalities [65,66]. Immature cytokinesis immediately following the first meiotic division without entering into the second meiotic division, or if polar body is re-wrapped into the female pronucleus, could produce unreduced gametes; it also plays a role that the sister chromatids do not separate or the second polar body cannot efflux normally in the second meiotic division. In the hybridization of red crucian carp×blunt snout bream, the triploid hybrids resulted from the retention of the second polar body of the fertilized eggs [60].

Polyspermy means the fertilization of an egg by two or more sperm nuclei [57]. It has been observed to induce polyploidy in some orchids, and is the most common mechanism leading to human triploids [31]. However, it is generally regarded as an uncommon mechanism of polyploid formation.

### 3.2 Polyploidy promoted by hybridization

In the process of species formation, hybridization and gene flow (introgression) between species is a regular occurrence, especially in rapidly radiating groups [11,60,67,68]. Mallet pointed out that hybridization would be a catalyst not only for speciation, but also for major evolutionary innovations [69]. Somatic chromosome doubling in hybrid offspring probably results in allopolyploidy [69]. Chromosome pairing often fails during meiosis in hybrid diploids, and the hybrids usually overcome this problem by producing unreduced gametes [1]. The formation and fusion of unreduced gametes accelerate the generation of polyploids from hybrid lineages.

Recent studies have indicated that whole genome duplication favors evolution of organisms. We believe that hybridization, especially distant hybridization, is an important driver of polyploidy accompanying genome duplication.

Fishes are the largest group of vertebrates, with plenty of opportunity for distant hybridization to occur. We have investigated the chromosome number of more than 300 fish species [70–72] and found that the majority of chromosomal numbers are even, especially 44, 48, 50, and 100 (Figure 1, see Appendix Table in the Chinese Version). Approximate ploidy relationships exist between many pairs of species, and many of the species are polyploids or have gone through polyploidization and distant hybridization during species formation.

Distant hybridization can promote genetic variation in the progeny, and produce fertile offspring, resulting in the formation of distant hybrid strains. The breeding of bisexual fertile allotetraploid fishes from the distant hybrid strains is important. The formation of the different ploidy fishes mainly depends on the genetic relationship between the parents [60]. Diploid or triploid hybrids were more likely to be formed than tetraploid hybrids in the first generation of the distant hybridization, when the parents had the same chromosome number. Nevertheless, breeders hope to produce tetraploid fish by building genetic lines from the diploid hybrids. On the other hand, in the first generation produced by distant hybridization where the parents had different chromosomal numbers, tetraploid hybrids, triploid hybrids, or natural diploid gynogenetic fish were more likely to be formed than diploid hybrids.

### 3.3 Parthenogenesis and "triploid bridge"

There are many parthenogenetic polyploids in animals [54–56]. During early evolution, unreduced eggs produced by distant hybridization could develop into diploid groups by parthenogenesis. The parthenogenetic diploids might generate unreduced eggs, whose combination with haploid sperm would form triploids. The triploids maintain their fertility by parthenogenesis, and their backcrosses to diploids could produce tetraploids [50]. This indirect route of tetraploid formation, using the triploid as a ladder, is referred to as a "triploid bridge" [10,69]. High ploidy populations could have evolved from the tetraploids. Higher polyploidy plants can also be produced through the "triploid bridge" [73]. For example, 1% tetraploid progeny were obtained by backcrossing a spontaneous triploid clone of *Populus tremula* to a diploid. Similarly, a small number of tetraploid progenies were obtained from triploid apple varieties that had themselves originated as spontaneous polyploids [57].

### 3.4 Organisms' habits can promote the occurrence of polyploids

Growth habits and breeding systems of species can also affect the production of unreduced gametes [57]. The proportion of polyploids is higher in perennial herbs with vegetative reproduction. Perhaps the presence of vegetative reproduction decreases the selection pressure to sexual reproduction, thus providing a relaxed environment for the generation of unreduced gametes and other nonfunctional gametes, further promoting the formation of unreduced gametes.

### 3.5 Environmental factors influence the occurrence of polyploids

The effect of the environment, especially temperature, on

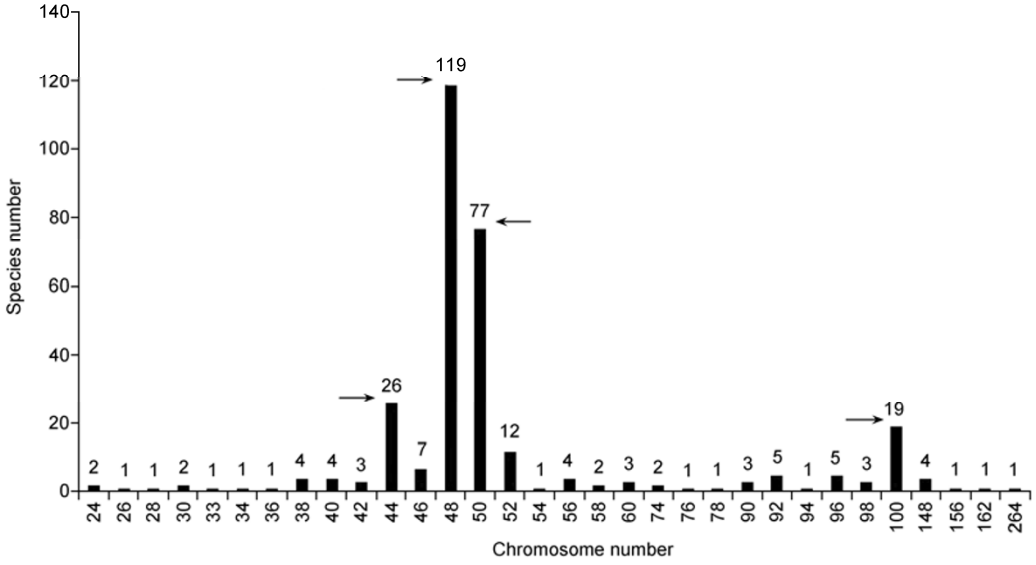


**Figure 1** Fish (318 species) chromosome number distribution (arrows indicate four types with the widest range).

polyploid formation cannot be ignored [31]. Environmental factors, such as cold and heat stimulation, and radiation, acting on diploid fertilized eggs, can promote chromosome doubling; when acting on meiosis of diploids, they can inhibit the efflux of the polar body, leading to the formation of polyploids. In the southeast rimland and nearby areas of the Qinghai-Tibet Plateau, the plateau uplift brought about a rapid change of temperature and humidity, becoming the major region for the generation of polyploid fish in China [50]. Among plants, a dramatic increase in 2*n* pollen production of *Uvularia grandiflora* following aberrant cold spells was observed [74]. In *Solanum tuberosum*, the ability of two genotypes to adapt to different temperatures by generating 2*n* gametes differs by a factor of two [75].

## 4 The genetic effects of polyploidy

### 4.1 Changes to cell and body size

Accompanying genome doubling and increases in genetic materials, the cell volumes of polyploids usually enlarge. Plants and animals may employ different strategies to cope with the increase in cell size associated with polyploidy [38,76]. Polyploid plants maintain the same number of cells as diploids and thus develop larger organs and body sizes. By contrast, many animal polyploids reduce the overall number of cells and maintain a similar organ and body size to their diploid progenitors.

### 4.2 Genomic change after polyploid formation

Genomic instability and genomic rapid recombination are the most characteristic features of the new polyploid, in an effort to achieve the harmonious co-existence of multiple genomes within one nucleus [77,78]. For instance, in artificial synthetic polyploid *Brassica* hybrids, extensive genomic rearrangements and fragment loss within five generations were observed [79]. The structural changes to the genome consist of deletion, insertion, duplication, translocation, and transposition [78]. Using chromosome painting techniques, Kenton *et al*. found at least nine intergenomic chromosomal rearrangements in allotetraploid tobacco (*Nicotiana tabacum*) [80]. Similarly, by genomic *in situ* hybridization (GISH), Jellen *et al*. detected five intergenomic translocations in allotetraploid oat (*Avena*), and approximately 18 such rearrangements in the allohexaploid [81]. Liu *et al*. [60] confirmed that the allotetraploid offspring of red crucian carp×common carp possess the parental genes, and demonstrated the hybridity by fluorescence *in situ* hybridization (FISH). Furthermore, genome recombination was found in allotetraploids at both the genome and transcriptome level, and it is hypothesized that the autosyndesis and allosyndesis of chromosomes coexist in meiosis of the germ cells (data to be published).

### 4.3 Changes in gene expression in polyploids

In addition to the genomic structural changes, polyploids also go through changes in gene expression [82–84], such as gene silencing, up-regulation or down-regulation of expression, nonfunctionalization, subfunctionalization, and neofunctionalization. Genetic and epigenetic mechanisms both play important roles [85]. Genetic changes are based on alteration of the DNA sequence, resulting in permanent changes in DNA sequence or gene loss. Epigenetic changes comprise DNA methylation, histone modification, RNA interference, and dosage compensation, which influence gene expression and display significant phenotypic effects [86]. After treatment with an inhibitor of DNA methyltransferase, Madlung *et al*. observed the development of altered morphologies in a synthetic *Arabidopsis* allotetraploid, and showed that DNA demethylation induced changes to the transcriptome, demonstrating the epigenetic regulation of expression in an allopolyploid [87]. In addition, the repression and activation of transposable elements can also cause changes in gene expression, and even promote rapid genome reorganization after polyploidization [88].

### 4.4 Diploidization

To maintain the stability after genome merging and doubling, the allopolyploid often performs diploidization, to eliminate a wide range of incompatibilities [89]. Cytologically, because the homoeologous chromosomes may pair during meiosis, which could prohibit the formation of functional gametes, allopolyploids often exhibit bivalent pairing of chromosomes rather than multivalent pairing, as a diploid-like meiotic behavior [1]. Special factors, such as the *Ph1* gene, are responsible for disomic inheritance in allopolyploid wheat. *Ph1* acts as a “local editor” to ensure specific centromere association between homologous chromosomes [90]. The synthetic *Arabidopsis* allotetraploids were capable of homologous pairing as early as three generations after their formation [91]. It is likely that either the parents provided genes controlling pairing behavior or features of the parental chromosomes hindered homoeologous pairing. Moreover, genomic sequence elimination, as well as chromosome rearrangement, also benefits cytological diploidization [91], as has been demonstrated in many polyploidy plants. In autopolyploids, the chromosome rearrangement, gene sequence changes, and consequently genetic changes may increase pairing fidelity over time, ultimately yielding disomic inheritance [1]. Genetically, diploidization refers to the phenomenon whereby the expression level of genes in an allopolyploid is reduced to a level comparable to its diploid progenitors, to avoid redundancy of duplicated genes [91]. The presence of many transposable elements and repetitive sequences, DNA methylation, and other epigenetic modifications can promote changes in gene expression and functional diversity in polyploids, thus accelerating the

process of diploidization [10]. Genetic and functional diploidization eases the slow transition from polyploids to new diploids. Many questions remain regarding diploidization, which require further research. Studies on artificial polyploids have already indicated their diploidization process, but whether or not these polyploids will change into diploids is unknown. In a word, the diploidization behavior of polyploids ensures their fertility and stability, in favor of survival and propagation.

### 4.5 Changes promote the evolution of polyploids

The abundant gene redundancy shields polyploids from the deleterious effects of mutation. Polyploids can mask recessive alleles by expressing dominant wild-type alleles, and diversify gene function by altering redundant copies of important or essential gene [11]. Furthermore, by fixing of different genomes, hybrid polyploids can integrate good parental traits and further display heterosis, showing better environmental adaptability compared with closely related diploid organisms [92]. By fast or slow genomic and gene expression changes, polyploids innovate and improve their function to achieve speciation and evolutionary success.

## 5 Polyploid breeding

### 5.1 Application of polyploid breeding in plants

Studies on the growth and biochemical characters of natural polyploid plants showed that polyploids, especially allopolyploids, have many obvious advantages, such as bigger nutritive organs, faster metabolism, more secondary metabolites, and increased stress resistance [93]. Artificial polyploid breeding is becoming more and more common in plants. For vegetable crops [33], fruit trees [94], horticultural plants [95], medicinal plants [96], by the application of artificial selective breeding, distant hybridization, tissue culture, physicochemical factors induced breeding, protoplast culture, and somatic hybridization, many polyploid lines were created and widely applied.

The triploid seedless watermelon is one of the best applications of polyploid breeding [97]. The key to cultivating triploid watermelon is the acquisition of tetraploid parents. The most widely used method is by colchicine-treatment. When treating the seeds and seedlings of diploid watermelon, colchicine hinders the formation of spindles. Thus, the genome is doubled and tetraploid watermelons are produced. The triploid seeds were obtained by crossing tetraploid (♀) with diploid (♂). The triploid plants cannot generate seeds because of meiotic abnormalities, while the ovary can develop into triploid fruit by the stimulation of diploid pollen. By integrating the dual advantages of polyploidization and hybrid origin, the triploid seedless watermelons gain obvious advantages, such as higher sugar content, increased stress resistance, higher yield, larger fruit, which are valued by the growers and consumers. China is now the biggest producer of seedless watermelon.

Many other polyploid lines have been obtained by selective breeding and hybridization in *Malus* (X=17), *Pyrus* (X=17), *Euvitis* (X=19), *Prunus* (X=8), and *Fragria* (X=7) [94]. By inducing chromosome duplication with artificial induction, tissue culture, and hybridization, polyploid cabbage (*Brassica rapa pekinensis*), lettuce (*Lactuca sativa*), capsicum (*Chili pepper*), lily (*Lilium brownie*), and cucumber (*Cucumis sativus*) were obtained, some of which have been widely planted and applied [33]. Among horticultural plants, polyploid common calla (*Zantedeschia aethiopica*), *Dendrobium devonianum*, *Armeniaca mumeBeauty mei*, balsamine (*Impatiens balsamina*), *Cymbidium goeringii*, mandala (*Datura stramonium*) were acquired [95]. Polyploid medicinal plants with higher output and increased stress resistance were formed in *Savia miltiorrhiza*, *Isatis tinctoria*, *Lonicera japonica*, *Glycyrrhiza pallidilora*, *Radix angelicae dahuricae*, and *Twotooth achyranthes* [96].

### 5.2 Application of polyploid breeding in animals

Studies on animal polyploid breeding have played a significant role in the field of aquatic organisms, and the breeding of polyploids in crustacea, shellfish, marine fish, and freshwater fish has made tremendous progress. Many kinds of polyploids were obtained by biological (distant hybridization, nuclear transfer, cell fusion), physical (temperature shock, hydrostatic pressure shock), or chemical (chemical inducers such as cytochalasin, caffeine, polyethylene glycol and colchicine) methods [98]. Among the polyploid animals, sterile triploids could translate the energy of gonad development into increased energy for flesh development, and display obvious advantages in growth rate, taste, survival rate, and disease resistance [99]. Moreover, triploids are not only a good way to control the density of the cultured fish, but also an effective approach to safeguard fish genetic resources [100]. There were two main pathways to produce triploid fish. One is direct induction and the other is indirect production by crossing a tetraploid with a diploid.

Artificial induction of aquatic organism polyploids by physical and chemical methods is based on the retention of the first or the second polar body of the oocytes, or the inhibition of the first cleavage of the fertilized eggs, thus producing a triploid or tetraploid [101].

Polyploid breeding in crustacea and shellfish often makes use of cytochalasin or temperature shock treatment, which generally cause high mortality at the early embryo stage or during larva development [102,103]. As a result of this mortality, large-scale production by these methods is difficult. Temperature shock and hydrostatic pressure shock have been used to induce polyploid fish. Song *et al.* used heat shock to treat yellow sang fish (*Pelteobagrus fulvidraco*) and gained 57% triploids [104]. Gui *et al.* successfully

achieved a triploid *C. auratus* transparent colored variety by hydrostatic pressure shock, and further obtained tetraploid embryos by a combination of hydrostatic pressure shock and cold shock [105]. These methods are subject to the control of treatment conditions and sample size, and cannot guarantee the production of 100% triploid offspring. While suppressing the first mitosis of the fertilized eggs, there have been no reports on the formation of bisexual fertile tetraploids. Acquisition of each generation of tetraploid broodstock must be handled manually, which increases costs.

However, treatments by physical and chemical factors are different from the conditions in nature, which may result in aneuploids. The chemical agents used to induce polyploids often react with genetic materials or other structures in the cytoplasm, influencing the individual's development and activity, and may produce malformation and chimeras.

Hybridization in nature could speed up species formation. Artificial hybridization in fish corresponds with natural evolutionary law, and accelerates the formation of polyploid groups. It has been proved that distant hybridization is the most widely used and effective biological method to produce allopolyploid fish. Wu *et al.* obtained several allotetraploids from the distant hybridization progeny of grass carp (*Ctenopharyngodon idellus*) and common carp [106]. Gui *et al*. found compound tetraploid allogynogenetic silver crucian carp in an artificial population [107]. Wu *et al*. acquired an artificial composite triploid by crossing the hybrids of xingguo red carp (*Cyprinus carpio* var.)×red crucian carp with scattered scales mirror carp (*C. carpio haematopterus*), but only the females were fertile [108]. Although these studies did not form bisexual fertile tetraploid populations, nor were put into production or application, they provided vital reference data for polyploid breeding by distant hybridization.

In our previous study, we found partial fertile diploid individuals in the first generation of the distant cross between red crucian carp and common carp. The $F_2$ hybrids obtained by self-crossing of the $F_1$ were all diploids. It is interesting that the males and females of diploid $F_2$ hybrids were able to generate unreduced diploid spermatozoa and diploid eggs, respectively, which were fertilized to form the allotetraploid hybrids ($4n$=200 or $2n$=4X=200, abbreviated as the $4n$AT) in the $F_3$ [60,62]. Until now, the $4n$AT has propagated to $F_{21}$, forming a stable tetraploid population. The bisexual fertile allotetraploid population with stable genetic traits provides adequate parents for the mass production of sterile triploids. The sterile triploid hybrids ($3n$=150, or $2n$=3X=150) were produced on a large scale by crossing the males of the $4n$AT with the females of diploid crucian carp or common carp. They were widely cultured in China and readily accepted by fish cultivators, and provided significant economic, social, and ecological benefits. Additionally, all-female triploid crucian carp were acquired by mating the allotetraploid females with sex reversal Japanese crucian carp (*Carassius cuvieri*). The all-female triploids are the first examples of polyploid fish obtained through the combination of sex reversal, gynogenesis, and diploid-tetraploid hybridization [109]. The bisexual fertile, genetically stable allotetraploid population, maintains the inheritance of heterosis and will perhaps become a new species [60]. Its generation provides an excellent model system for exploring the nature of polyploid origin and the evolution of fish.

The development of transgenic fish has made substantial advances in China [110,111]; however, because of the possible ecological risk posed by these fish, worldwide transgenic fish are not at the stage of commercialization. By crossing improved tetraploid red crucian carp hybrids with diploid transgenic yellow river carp that possessed an additional grass carp growth hormone gene (*GCGH*), a transgenic triploid fish was developed [112], which possessed some beneficial characteristics, such as faster growth rate and higher utilization rate of forage. Hybridization between different ploidy could yield 100% sterile triploid progeny, thus solving the problem of the ecological safety of transgenic fish. This method of producing transgenic triploid fish could also guarantee the supply of individuals for commercial aquaculture.

Distant hybridization of red crucian carp (♀) and blunt snout bream (♂), a combination between the parents with different chromosomal number, has also made great progress [59,113]. In the first generation, sterile triploid hybrids ($3n$=124, or $2n$=3X=124) and bisexual fertile tetraploid hybrids ($4n$=148, or $2n$=4X=148) were obtained. Tetraploid hybrids can generate both reduced and unreduced gametes; therefore, the self-mating and breeding of the tetraploid hybrids formed a tetraploid clonal line ($4n$=200, or $2n$=4X=200). By mating the tetraploid clonal line with diploid crucian carp, a new kind of triploid hybrid ($3n$=150, or $2n$=3X=150) was produced. The tetraploid eggs of the first generation of tetraploid hybrids ($4n$=148) were fertilized with haploid sperm of the blunt snout bream and red crucian carp, respectively, to form two kinds of pentaploid hybrids ($5n$=172 or $5n$=198), the first of their kind in vertebrates.

## 6 Conclusion and outlook

Through the in-depth study of polyploid organisms, researchers have suggested that polyploidization played an important role in the history of biological evolution. Traditional studies mainly made use of artificial allopolyploidy model plants and crop plants, such as *Brassica*, *Arabidopsis*, wheat (*Triticum*), cotton (*Gossypium*), corn (*Zea mays*), and tobacco (*Nicotiana*), and established the understanding of genomic and genetic attributes following polyploidization. Currently, with the rapid development of genome sequencing, and modern molecular biological and bioinformatics techniques, studies on wild polyploids are increasing.

However, researchers hold different views regarding the exact time of polyploidy, the frequency, rate, and ploidy times of different organisms. Therefore, more studies on more species are required, especially the polyploidy model animals, to solve the above problems.

Research advances on polyploid animals is relatively slow, because of the lack of model species. Although there has been good progress in the study of epigenetic influence on genotype and phenotype of polyploidy model plants, there are fewer relevant epigenetic studies on polyploid animals. The allotetraploid clonal line of red crucian carp×common carp and the polyploid clonal line of red crucian carp×blunt snout bream undoubtedly provide good models for research into polyploid animals. The on-going work using BAC (bacterial artificial chromosome) library construction, transcriptome sequencing, methylation assay, identification of *Sox* gene clusters and *Hox* gene clusters, and whole genome sequencing, will provide powerful data and theoretical support for the study of animal polyploidization.

Although there are many studies on polyploidization, the details of the mechanisms of polyploid formation require further in-depth study. There is no doubt that continuing studies of polyploid plants and animals are of great significance in biological evolution, genetics, and breeding.

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1 Otto S P. The evolutionary consequences of polyploidy. Cell, 2007, 131: 452–462
2 Ohno S. Evolution by Gene Duplication. New York: Springer-Verlag, 1970
3 Holland P W H, Garcia-Fernàndez J, Williams N A, *et al*. Gene duplications and the origins of vertebrate development. Development (Supplement), 1994, 125–133
4 Meyer A, Van de Peer Y. From 2R to 3R: evidence for a fish-specific genome duplication (FSGD). Bioessays, 2005, 27: 937–945
5 Dehal P, Boore J L. Two rounds of whole genome duplication in the ancestral vertebrate. PLoS Biol, 2005, 3: 1700–1708
6 Blomme T, Vandepoele K, De Bodt S, *et al*. The gain and loss of genes during 600 million years of vertebrate evolution. Genome Biol, 2006, 7: R43
7 Amores A, Force A, Yan Y L, *et al*. Zebrafish *hox* clusters and vertebrate genome evolution. Science, 1998, 282: 1711–1714
8 Taylor J S, Van de Peer Y, Braasch I, *et al*. Comparative genomics provides evidence for an ancient genome duplication event in fish. Philos Trans R Soc, 2001a, 356: 1661–1679
9 Volff J N. Genome evolution and biodiversity in teleost fish. Heredity, 2005, 94: 280–294
10 Soltis D E, Soltis P S, Tate J A. Advances in the study of polyploidy since plant speciation. New Phytol, 2003, 161: 173–191
11 Comai L. The advantages and disadvantages of being polyploid. Nat Rev Genet, 2005, 6: 836–845
12 Kassahn K S, Dang V T, Wilkins S J, *et al*. Evolution of gene function and regulatory control after whole-genome duplication: comparative analyses in vertebrates. Genome Res, 2009, 19: 1404–1418
13 Soltis D E, Soltis P S. Molecular data and the dynamic nature of polyploidy. Crit Rev Plant Sci, 1993, 12: 243–273
14 Grant V. Plant Speciation, 2nd ed. New York: Columbia University Press, 1981
15 Ahuja M R, Neale D B. Evolution of genome size in conifers. Silvae Genet, 2005, 54: 126–137
16 Hair J B. The chromosomes of the Cupressaceae. I. Tetraclineae and Actinostrobeae (Callitroideae). New Zeal J Bot, 1968, 6: 277–284
17 Gates R R. The stature and chromosomes of *Oenothera gigas* De Vries. Arch F Zellforsch, 1909, 3: 525–552
18 Goldblatt P. Polyploidy in Angiosperms: Monocotyledons. In: Lewis W H, ed. Polyploidy: Biological Relevance. New York: Plenum Press, 1980. 219–239
19 Lewis W H. Polyploidy in Angiosperms: Dicotyledons. In: Lewis W H, ed. Polyploidy: Biological Relevance. New York: Plenum Press, 1980. 241–268
20 Masterson J. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science, 1994, 264: 421–423
21 Bowers J E, Chapman B A, Rong J K, *et al*. Unravelling angiosperm genome evolution by phylogenetic analysis of chromosomal duplication events. Nature, 2003, 422: 433–438
22 Soltis D E, Albert V A, Leebens-Mack J, *et al*. Polyploidy and angiosperm diversification. Am J Bot, 2009, 96: 336–348
23 Veron A S, Kaufmann K, Bornberg-Bauer E. Evidence of interaction network evolution by whole-genome duplications: a case study in MADS-box proteins. Mol Biol Evol, 2007, 24: 670–678
24 Albert V A, Soltis D E, Carlson J E, *et al*. Floral gene resources from basal angiosperms for comparative genomics research. BMC Plant Biol, 2005, 5: 5–16
25 Cui L, Wall P K, Leebens-Mack J, *et al*. Widespread genome duplications throughout the history of flowering plants. Genome Res, 2006, 16: 738–749
26 Zhang F W, Wang Y R. Progress of polyploidy breeding technology applied in medical plants scale (in Chinese). Guiding J TCM, 2006, 12: 83–85
27 Paterson A H, Bowers J, Burow M, *et al*. Comparative genomics of plant chromosomes. Plant Cell, 2000, 12: 1523–1539
28 Severin A J, Cannon S B, Graham M M, *et al*. Changes in twelve homoeologous genomic regions in soybean following three rounds of polyploidy. Plant Cell, 2011, 23: 3129–3136
29 Jaillon O, Aury J M, Noel B, *et al*. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. Nature, 2007, 449: 463–467
30 Ming R, Hou S, Feng Y, *et al*. The draft genome of the transgenic tropical fruit tree papaya (*Carica papaya* Linnaeus). Nature, 2008, 452: 991–996
31 Otto S P, Whitton J. Polyploid incidence and evolution. Annu Rev Genet, 2000, 34: 401–437
32 Liu Z D. Yichuanxue (in Chinese). Beijing: Higher Education Press, 1991
33 Yang Y G, Zhuang Y, Chen L Z, *et al*. Vegetable polyploid and polyploidy breeding (in Chinese). Acta Agricult Univ Jiangxi, 2006, 28: 534–538
34 Hilu K W. Polyploidy and the evolution of domesticated plants. Am J Bot, 1993, 80: 1494–1499
35 Fedorov A. Chromosome Numbers of Flowering Plants. Leningrad: Acad Sci USSR Komarov Botanical Institute, 1969
36 Dai S L, Wang W K, Huang J P. Advances of researches on phylogeny of *Dendranthema* and origin of chrysanthemum (in Chinese). J Beijing Forest Univ, 2002, 24: 230–234
37 Jin X X, Zhang Q X. Advances in the studies of breeding *Primula* (in Chinese). Chin Bull Bot, 2005, 22: 738–745
38 Gregory T R, Mable B K. Polyploidy in Animals. In: Gregory T R, ed. The Evolution of the Genome. San Diego: Elsevier, 2005. 427–517
39 Wu M. Genetics and evolution of animal polyploid (in Chinese). Chin J Zool, 1988, 23: 48–51

40 Chen D W, Daoye M Y. Chromosomes and systematic classification of molluscs. Chin J Zool, 1988, 23: 48–51
41 Ye M W. The polyploidy phenomenon and formation in animals and plants (in Chinese). Bull Biol, 1998, 33: 21–23
42 Li S W. Polyploid insects (in Chinese). Entomol Knowledge, 2002, 39: 147–151
43 Naruse K, Tanaka M, Mita K, *et al*. A medaka gene map: the trace of ancestral vertebrate proto-chromosomes revealed by comparative gene mapping. Genome Res, 2004, 14: 820–828
44 Woods I G, Wilson C, Friedlander B, *et al*. The zebrafish gene map defines ancestral vertebrate chromosomes. Genome Res, 2005, 15: 1307–1314
45 Gui J F, Zhou L. Genetic basis and breeding application of clonal diversity and dual reproduction modes in polyploid *Carassius auratus gibelio*. Sci China Life Sci, 2010, 53: 409–415
46 Zhou L, Gui J F. Karyotypic diversity in polyploid gibel carp, *Carassius auratus gibelio* bloch. Genetica, 2002, 115: 223–232
47 Xiao J, Zou T M, Chen Y B, *et al*. Coexistence of diploid, triploid and tetraploid crucian carp (*Carassius auratus*) in natural waters. BMC Genet, 2011, 12: 20
48 Luo J R. Polyploid fishes and fish polyploidy breeding (in Chinese). Pearl River Fisheries, 1991, 17: 69–74
49 Lampert K P, Schartl M. The origin and evolution of a unisexual hybrid: *Poecilia formosa*. Philos Trans R Soc Lond B Biol Sci, 2008, 363: 2901–2909
50 Zan R G. The polyploids in fish and their roles in fish evolution (in Chinese). J Yunnan Univ, 1985, 7: 235–243
51 Abbas K, Li M Y, Wang W M, *et al*. First record of the natural occurrence of hexaploids loach *Misgurnus anguillicaudatus* in Hubei Province, China. J Fish Biol, 2009, 75: 435–441
52 Ráb P, Rábová M, Bohlen J, *et al*. Genetic differentiation of the two hybrid diploid-polyploid complexes of loaches, genus *Cobitis* (Cobitidae) involving *C. taenia*, *C. elongatoides* and *C.* spp. in the Czech Republic: karyotypes and cytogenetic diversity. Folia Zool, 2000, 49: S55–S66
53 Boroň A, Kotusz J. The preliminary data on diploid-polyploid complexes of the genus *Cobitis* in the Odra River basin, Poland (Pisces, Cobitidae). Folia Zool, 2000, 49: S79–S84
54 Li S S. Amphibians's chromosomes and their evolution (in Chinese). Chin J Zool, 1991, 26: 47–52
55 Li S S. Parthenogenesis in reptiles (in Chinese). Chin J Zool, 1992, 27: 41–44
56 Li S S. Vertebrate's polyploid (in Chinese). Chin J Zool, 1980, 2: 52–54
57 Ramsey J, Schemske D W. Pathways, mechanisms, and rates of polyploid formation in flowering plants. Annu Rev Ecol Syst, 1998, 29: 467–501
58 Newton W C F, Pellew C. *Primula kewensis* and its derivatives. J Genet, 1929, 20: 405–467
59 Liu S J, Qin Q B, Xiao J, *et al*. The formation of the polyploid hybrids from different subfamily fish crossing and its evolutionary significance. Genetics, 2007, 176: 1023–1034
60 Liu S J. Distant hybridization leads to different ploidy fishes. Sci China Life Sci , 2010, 53: 416–425
61 Karpechenko G D. The production of polyploid gametes in hybrids. Hereditas, 1927, 9: 349–368
62 Liu S J, Liu Y, Zhou G J, *et al*. The formation of tetraploid stocks of red crucian carp × common carp hybrids as an effect of interspecific hybridization. Aquaculture, 2001, 192: 171–186
63 Zhang C, Sun Y D, Liu S J, *et al*. Evidence of the unreduced diploid eggs generated from the diploid gynogenetic progeny of allotetraploid hybrids (in Chinese). Acta Genet Sin, 2005, 32: 136–144
64 Ullah Z, Lee C Y, DePamphilis M L. Cip/Kip cyclin-dependent protein kinase inhibitors and the road to polyploidy. Cell Div, 2009, 4: 10
65 Bretagnolle F, Thompson J D. Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. New phytol, 1995, 129: 1–22
66 Werner J E, Peloquin S J. Occurrence and mechanisms of 2*n* egg formation in 2x potato. Genome, 1991, 34: 975–982
67 Seehausen O. Hybridization and adaptive radiation. Trends Ecol Evol, 2004, 19: 198–207
68 Mallet J. Hybridization as an invasion of the genome. Trends Ecol Evol, 2005, 20: 229–237
69 Mallet J. Hybrid speciation. Nature, 2007, 446: 279–283
70 Yu X J, Zhou T, Li Y C. Chromosomes of Chinese Fresh-water Fishes (in Chinese). Beijing: Science Press, 1989
71 Meyer A, Salzburger W, Schartl M. Hybrid origin of a swordtail species (Teleostei: *Xiphophorus clemenciae*) driven by sexual selection. Mol Ecol, 2006, 15: 721–730
72 Saitoh K, Chen W J, Mayden R L. Extensive hybridization and tetrapolyploidy in spined loach fish. Mol Phylogenet Evol, 2010, 56: 1001–1010
73 Harlan J R, deWet J M J. On Ö. Winge and a prayer: the origins of polyploidy. Bot Rev, 1975, 41: 361–390
74 Belling J. The origin of chromosomal mutations in *Uvularia*. J Genet, 1925, 15: 245–266
75 McHale N A. Environmental induction of high frequency 2*n* pollen formation in diploid *Solanum*. Can J Genet Cytol, 1983, 25: 609–615
76 Mable B K. 'Why polyploidy is rarer in animals than in plants': myths and mechanisms. Biol J Linn Soc, 2004, 82: 453–466
77 Comai L. Genetic and epigenetic interactions in allopolyploid plants. Plant Mol Biol, 2000, 43: 387–399
78 Chen Z J, Ni Z F. Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. Bioessays, 2006, 28: 240–252
79 Song K, Lu P, Tang K, *et al*. Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proc Natl Acad Sci USA, 1995, 92: 7719–7723
80 Kenton A, Parokonny A S, Gleba Y Y, *et al*. Characterization of the *Nicotiana tabacum* L. Genome by molecular cytogenetics. Mol Gen Genet, 1993, 240: 159–169
81 Jellen E N, Gill B S, Cox T S. Genomic *in situ* hybridization differentiates between A/D-and C-genome chromatin and detects intergenomic translocations in polyploid oat species (genus *Avena*). Genome, 1994, 37: 613–618
82 Kellogg E A. What happens to genes in duplicated genomes. Proc Natl Acad Sci USA, 2003, 100: 4369–4371
83 Se´mon M, Wolfe K H. Preferential subfunctionalization of slow-evolving genes after allopolyploidization in *Xenopus laevis*. Proc Natl Acad Sci USA, 2008, 105: 8333–8338
84 Lee H S, Chen Z J. Protein-coding genes are epigenetically regulated in *Arabidopsis* polyploids. Proc Natl Acad Sci USA, 2001, 98: 6753– 6758
85 Chen Z J. Genetic and epigenetic mechanisms for gene expression and phenotypic variation in plant polyploids. Annu Rev Plant Biol, 2007, 58: 377–406
86 Liu B, Wendel J F. Epigenetic phenomena and the evolution of plant allopolyploids. Mol Phylogenet Evol, 2003, 29: 365–379
87 Madlung A, Masuelli R W, Watson B, *et al*. Remodeling of DNA methylation and phenotypic and transcriptional changes in synthetic *Arabidopsis* allotetraploids. Plant Physiol, 2002, 129: 733–746
88 Fedoroff N. Transposons and genome evolution in plants. Proc Natl Acad Sci USA, 2000, 97: 7002–7007
89 Doyle J J, Flagel L E, Paterson A H, *et al*. Evolutionary genetics of genome merger and doubling in plants. Annu Rev Genet, 2008, 42: 443–461
90 Liu B, Wendel J F. Non-mendelian phenomena in allopolyploid genome evolution. Curr Genomics, 2002, 3: 489–506
91 Ma X F, Gustafson J P. Genome evolution of allopolyploids: a process of cytological and genetic diploidization. Cytogenet Genome Res, 2005, 109: 236–249
92 De Bodt S, Maere S, Van de Peer Y. Genome duplication and the origin of angiosperms. Trends Ecol Evol, 2005, 20: 591–597
93 Ma H Y, Zhang J F, Li Z D. Research advances on plant polyploidy breeding techniques (in Chinese). Protect Forest Sci Technol, 2008, 1: 43–46

94 Wang T K, Zhang J Z, Qi Y S, *et al*. Advances on polyploid breeding of fruit crops in China (in Chinese). J Fruit Sci, 2004, 21: 592–597
95 Shun M H, Zhang S N. The application of polyploidy breeding in horticultural crops (in Chinese). Jiangsu Agricult Sci, 2004, 1: 68–72
96 Zhang X Y, Liu J F, Wang L P. Polyploidy breeding and its application research progress of medicinal plants (in Chinese). J Jilin Normal Univ (Nat Sci Ed), 2009, 4: 128–131
97 Yuan J M, Dang X M, Zhan Y F. Advances on polyploid breeding in watermelon (in Chinese). Chin J Tropical Agricult, 2009, 29: 65–70
98 Shen A L, Yao W Z. The proceeding on triploid breeding of aquatic animals (in Chinese). Reserv Fish, 2004, 24: 1–3
99 Liu Y, Liu S J, Sun Y D, *et al*. Polyploid hybrids of crucian carp× common carp (in Chinese). Rev China Agricult Sci Technol, 2003, 5: 3–6
100 Wu P. Research progress of fish polyploid breeding in China (in Chinese). J Shanghai Fish Univ, 2005, 14: 72–78
101 Hu L L, Li J E. The review of fish polyploid breeding research(in Chinese). Fish Sci Technol, 2009, 7–10
102 Yuan B J, Jiang N C, Lu J P, *et al*. A review of decapod crustacean multiploid breeding (in Chinese). Donghai Marine Sci, 1998, 16: 64– 68
103 Wang Z P, Li K J, Yu R H, *et al*. Progress of tetraploid breeding in mollusks (in Chinese). J Ocean Univ China, 2004, 34: 195–200
104 Song L M, Yang Y, Wang W M, *et al*. Induction of triploidy in yellow catfish *Pelteobagrus fulvidraco* by heat shock (in Chinese). Fish Sci, 2010, 29: 352–355
105 Gui J F, Liang S C, Sun J M, *et al*. Studies on genome manipulation in fish I. Induction of triploid transparent colored crucian carp (*Carassius auratus* transparent colored variety) by hydrostatic pressure (in Chinese). Acta Hydrobiol Sin, 1990, 14: 336–344
106 Wu W X, Li C W, Liu G A, *et al*. Studies on tetraploid hybrid between red common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idellus*) and its backcross triploid (in Chinese). Acta Hydrobiol Sin, 1988, 12: 355–363
107 Gui J F, Liang S C, Zhu L F, *et al*. Discovery and breeding potential of compound tetraploid allogynogenetic silver crucian carp in artificial population (in Chinese). Chin Sci Bull, 1992, 37: 646–648
108 Wu C, Ye Y, Chen R, *et al*. An artificial multiple triploid carp and its biological characteristics. Aquaculture, 1993, 111: 255–262
109 Luo K K, Xiao J, Liu S J, *et al*. Massive production of all-female diploids and triploids in the crucian carp. Int J Biol Sci, 2011, 7: 487–495
110 Hu W, Zhu Z Y. Integration mechanisms of transgenes and population fitness of GH transgenic fish. Sci China Life Sci, 2010, 53: 401–408
111 Hu W, Wang Y P, Zhu Z Y. Progress in the evaluation of transgenic fish for possible ecological risk and its containment strategies. Sci China Life Sci, 2007, 50: 573–579
112 Yu F, Xiao J, Liang X Y, *et al*. Rapid growth and sterility of growth hormone gene transgenic triploid carp. Chin Sci Bull, 2011, 56: 1679–1684
113 Qin Q B, He W G, Liu S J, *et al*. Analysis of 5S rDNA organization and variation in polyploid hybrids from crosses of different fish subfamilies. J Exp Zool (Mol Dev Evol), 2010, 314: 403–411