

The many costs of sex

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Explaining the evolution of sex is challenging for biologists. A 'twofold cost' compared with asexual reproduction is often quoted. If a cost of this magnitude exists, the benefits of sex must be large for it to have evolved and be maintained. Focusing on benefits can be misleading, as this sidelines important questions about the cost of sex: what is the source of the twofold cost: males, genome dilution or both? Does the cost deviate from twofold? What other factors make sex costly? How should the costs of sex be empirically measured? The total cost of sex and how it varies in different contexts must be known to determine the benefits needed to account for the origin and maintenance of sex.

What is meant by the costs of sex, and why does it matter?

Sex (defined in Box 1) is an evolutionary puzzle. In several ways, sexual reproduction is a less efficient method of reproduction compared with asexual reproduction [1–7]. Consequently, most work on the evolution of sex looks for compensatory benefits of sex. This has resulted in estimates of the actual costs of sex being neglected. There is a tendency, especially in textbooks, to reference a single baseline estimate, by stating that sex imposes a twofold cost. The cost is often attributed to the production of males (females waste half of their resources on producing males, who in turn invest minimally in offspring [1,3]) or to genome dilution (only half of the parental genome is transferred to offspring [2]), or, confusingly, to both.

Before explaining the true source and the actual magnitude of the 'twofold' cost, a working definition of 'the costs of sex' is needed. We define this as the magnitude of the minimum compensatory benefits that enable sexual individuals to avoid being outcompeted by asexual individuals [4]. For example, if sex has an exactly twofold cost, then it would also have to have benefits of at least a twofold magnitude to persist in competition with asexuals. Ideally, this cost includes all fitness components that are reduced when a sexually reproducing individual is compared with its asexual counterpart.

Identifying costs is a major empirical challenge. Theoretical work can exclude benefits of sex [8,9] and simply quantify the cost as the fitness ratio of asexual to sexual organisms. Empirically, however, this does not work. If immediate benefits of sex exist, the fitness ratio will underestimate the cost aspect of the equation. Additionally, suitable asexual counterparts might not exist for a given sexual species. Researchers must be mindful of taxonomic variation in life histories and reproductive modes (e.g. unicellular

or multicellular; hermaphroditic or with distinct males and females), rather than simply assume a twofold cost of sex.

Is genome dilution responsible for the twofold cost of sex?

During the 1970s, George Williams [2] and John Maynard Smith [1,3] produced two theories that invoked an exactly twofold cost of sex owing to genome dilution and male production, respectively. Although it has been known since the late 1970s that genome dilution is not the source of the twofold cost of sex in species with distinct males and females [10–14], both explanations are still encountered in the literature. Genome dilution refers to all the genes of asexually produced offspring being from their single parent, whereas only half the genes in the offspring of a sexual female are from the mother. The representation of maternal genes is reduced by 50% each generation.

The error in the argument is that only the genes that determine the mode of reproduction matter in this context [10–12]. Diluting the rest of the genome is irrelevant: genes for sex are only diluted if the genes of one parent that code for sex are replaced by genes from the other parent that code for asexual reproduction. This does not easily occur because both parents must have genes for sex, otherwise they would not have mated. Although details differ for recessive and

Glossary

Amphimixis: sexual reproduction by the fusion of gametes from two organisms.

Androgenesis: reproduction in which diploid offspring carry nuclear chromosomes from the male parent only.

Anisogamy: a form of sexual reproduction in which the fusing gametes are of markedly unequal size. The sexes are defined according to anisogamy; the sex with the smaller gametes is defined as male. In the absence of anisogamy (isogamy), one speaks of mating types rather than separate sexes.

Apomixis: a subcategory of asexual reproduction, in which meiosis is suppressed and there is a single mitotic maturation division. The offspring are genetically identical to the mother.

Arrhenotoky: the production of males from unfertilised eggs (such as in haplodiploid systems found in Hymenoptera).

Automixis: a subcategory of asexual reproduction, in which meiosis is normal, producing four haploid pronuclei. The diploid number is restored by the fusion of two pronuclei or of two early cleavage nuclei. The offspring can be genetically different from, and more homozygous than the mother.

Dioecious: the botanical term for a species with separate sexes (see also gonochoric).

Gonochoric: the zoological term for a species with separate sexes (see also dioecious).

Gynogenesis: a reproductive system where a diploid egg is produced asexually, but requires contact with sperm before it begins development. Sperm do not contribute genes to the offspring genome.

Isogamy: a form of sexual reproduction in which the fusing gametes are of equal size (see also anisogamy).

Karyogamy: the fusion of two gametic nuclei.

Syngamy: the fusion of two gametes to form a zygote.

Thelytoky: a form of parthenogenesis where only female offspring are produced. In this review, asexual reproduction refers to thelytoky unless otherwise stated.

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Box 1. Sexual and asexual reproduction

Terminology and definitions surrounding sexual and asexual reproduction are used inconsistently in the literature, which can easily lead to confusion [20,60,61]. A full account of the various modes of sexual and asexual reproduction is beyond the scope of this review, but some definitions are required. Here, we define sexual reproduction (hereafter 'sex') as the union of two gametes and genomes. It includes species whose gametes are not morphologically differentiated (isogamy) so that two sexes (males and females) do not exist. If sperm and eggs exist, these can be produced by hermaphrodites or by gonochorists (separate sexes). We exclude genetic exchange in prokaryotes by horizontal gene transfer from our definition of sex.

Unless otherwise mentioned, by asexual reproduction we refer to thelytoky, a form of parthenogenesis where females produce offspring from unfertilised eggs, and all offspring are female. This is not the only possible form of asexuality, however, as illustrated by other cases in the empirical section of the main text (see also Box 3).

Asexual reproduction is sometimes incorrectly assumed to be synonymous with clonal reproduction. Two types of parthenogenesis can be distinguished according to how diploidy in offspring is achieved [61]. With apomixis, offspring are genetical clones of their mother because meiosis is suppressed. Heterozygosity and genetic diversity are preserved. With automixis, meiosis is not suppressed; instead diploidy is restored during or after meiosis. This can happen in several ways [20,60–62]. Automixis leads to increased homozygosity among offspring, similar to close inbreeding or selfing in sexual hermaphrodites. Unless otherwise stated, however, the general points in our review apply to both apomictic and automictic systems.

dominant genes (Box 2), in both cases genes for sex 'recognise' each other and 'reunite' in offspring because they cause sexual parents to mate with each other. These reunions negate the 50% dilution. If the 'recognition and reunite' process breaks down, however, gene dilution retains a net effect to make sex costly, as can occur in hermaphrodites and when asexual organisms still produce males [15–22] (Box 3).

Are males the culprit for the (sometimes) twofold cost of sex?

Anisogamous lineages produce two types of gamete. By definition [23], males produce the smaller type and females the larger type. The real twofold cost of sex is the cost of

producing males who inefficiently convert resources into offspring [1,3]. If a female invests half her reproductive resources into males that, in turn, invest minimally into each offspring sired then, all else being equal, asexuality is exactly twice as efficient at converting resources into descendants (Figure 1a versus 1c). Males spend resources to outcompete each other; only females directly invest resources into producing offspring. This cost of male production occurs even when genes for sex are fully maintained within a sexual lineage (no dilution, Box 2). Parthenogenesis should outcompete sex because it avoids diverting 50% of resources to males [1,3].

Is the cost of male production always twofold? No. Twofoldness assumes that males neither hinder nor assist females in offspring production and invest all their reproductive effort into maximising their number of fertilisations. When males play a zero-sum game over paternity, the total number of eggs remains unchanged, no matter how males compete. The cost of sex owing to males can deviate substantially from twofold if reproduction differs from this picture.

Consider an isogamous species with two mating types, where gametic resources are the only form of parental investment. If both types invest equally into gametes, sex may cause no decline in reproductive output compared with an asexual lineage (Figure 1a versus 1b). The same logic applies to postzygotic paternal care. If males care for offspring or, for example, transfer nutritious spermatozoa to females, females can produce larger broods or reproduce faster. Females still have to produce males, but the cost is less than twofold [3]. This situation is most likely to occur when strict monogamy makes male and female interests coincide. Males should then evolve traits that maximise female reproductive success, minimising the cost of sex owing to males.

Conflict elevates the cost

Lifelong monogamy is rare in nature. The male-imposed cost on females increases with sexual conflict in mating

Box 2. When dilution is irrelevant: sex as a green beard

Consider a gonochoristic, obligately sexual species. Sexual individuals have two copies of the recessive allele *a*. A dominant mutant allele *A* induces apomictic parthenogenesis, and no male offspring are produced. Given that *A* is dominant, *Aa*s never mate, and *AA*s are never produced. When an *aa* individual mates, its partner must have an *aa* genotype, and so will their offspring. There is no genome dilution that allows the parthenogenesis gene to invade the sexual population. The sexual and asexual lineages are genetically isolated, as first noted by Treisman and Dawkins [10], Dawkins [12] and Barash [11]. However, the twofold cost of males [1,3] still applies (compare Figure 1a and 1c, main text).

The situation is more complex when a dominant allele *B* codes for sex. When two heterozygous sexual types (*Bb*) mate, 25% of their offspring are asexual (*bb*). Because *bb* individuals do not mate, their genes have no pathway back to the offspring of sexual individuals. No matter how successfully asexuals reproduce, elevating the prevalence of the *b* allele, this does not dilute the genetic composition of sexual offspring.

As long as heterozygotes exist, some offspring of sexuals will be asexual, but this is a transient state. From the viewpoint of the sexual population (*BB* and *Bb* genotypes), the *b* allele is like a lethal recessive allele. Recessive homozygotes are removed from the

sexual gene pool, similar to homozygotes for a lethal recessive. The *b* allele is eventually purged from the sexual population (ignoring mutation). This will again lead to genetic isolation between the asexual and sexual population (i.e. only *BB* sexual and *bb* asexual types) (see also [10]).

The greenbeard effect offers an analogy. Greenbeards are genes that identify the presence of copies of themselves in other individuals, and cause their bearer to behave nepotistically toward these individuals [63]. In our first example, the *a* allele is analogous to a greenbeard gene that 'identifies' other homozygous carriers simply because they are willing to have sex, and therefore 'chooses' them as mating partners. The parent forfeits one allele for sexual reproduction, but it is guaranteed to be replaced by another similar allele from the other parent. The analogy extends even further. Theory predicts the possibility of cheats ('falsebeards'), which display the 'label' for identification (here, willingness to have sex), without then being cooperative (by giving up one of its own alleles) [63]. This is analogous to gynogenesis and androgenesis, where an asexual individual superficially appears to participate in sexual reproduction, but passes on its entire genome to the offspring and discards the genes of its partner. Real-world examples include Amazon mollies *Poecilia formosa* (gynogenesis) [64] and clams *Corbicula spp* (androgenesis) [65].

Box 3. Genome dilution in the absence of a cost of male production

Consider a hermaphroditic species. Wild-type individuals are obligately sexual and carry two copies of the *a* allele. Mutant *Aa* individuals produce parthenogenetic eggs, but they also maintain their male function and continue to produce sperm via normal meiosis. This sperm can fertilise eggs of sexual (*aa*) individuals.

As *aa* and *Aa* individuals now both allocate equivalent amounts of resources to sperm production, there can be no cost of male function. Sperm of *Aa* individuals can contain the *A* allele. Such sperm is capable of fertilising the eggs of *aa* individuals, thereby using the resources of obligately sexual *aa* individuals to produce parthenogenetic *Aa* offspring. In this case, the wild-type and parthenogenetic lineages are not genetically isolated from each other (as they were in Box 2), leading to a cost of genome dilution being imposed upon the sexual lineage. The *AA* genotype is not produced: only *a*-eggs fuse with sperm.

The dynamics of the gene frequencies in this system are described by the recursion equations (Equations I):

$$\begin{cases} p_{aa}(t+1) = p_{aa}(t)^2 + 1/2 p_{Aa}(t) p_{aa}(t) \\ p_{Aa}(t+1) = p_{Aa}(t) + 1/2 p_{Aa}(t) p_{aa}(t) \end{cases} \quad (I)$$

When the *Aa* mutant initially appears in a large population, $p_{aa} \approx 1$, and therefore:

$$p_{Aa}(t+1) \approx p_{Aa}(t) + 1/2 p_{Aa}(t) = 3/2 p_{Aa}(t) \quad (II)$$

This implies that the *Aa* genotype initially has a 3/2-fold advantage. This advantage will diminish as p_{Aa} increases, but it can be shown that the frequencies converge towards $p_{Aa} = 1$, $p_{aa} = 0$. The advantage of the parthenogenetic genotype is therefore maintained at all frequencies and, all else being equal, will eventually invade the population despite the lack of any cost of male function. This cost of sex in hermaphrodites was first noted by Jaenike & Selander [15]. Equation I and, therefore, the same costs as in that example, can also be applied to gonochoristic species [20]. Here, *Aa* individuals are assumed to continue to produce males in addition to parthenogenetically produced female offspring.

In certain situations, an intermediate level of investment in male production, or male function can lead to the greatest fitness advantage over sexual individuals [18,19]. In these cases, the cost of males or male function and the cost of genome dilution can apply simultaneously, as parthenogenetic types invest less in male production than do sexual individuals.

We encourage readers to consider the costs of sex on a case-by-case basis, taking into account both the potential cost of males or male function and the genetics underlying the reproductive mode.

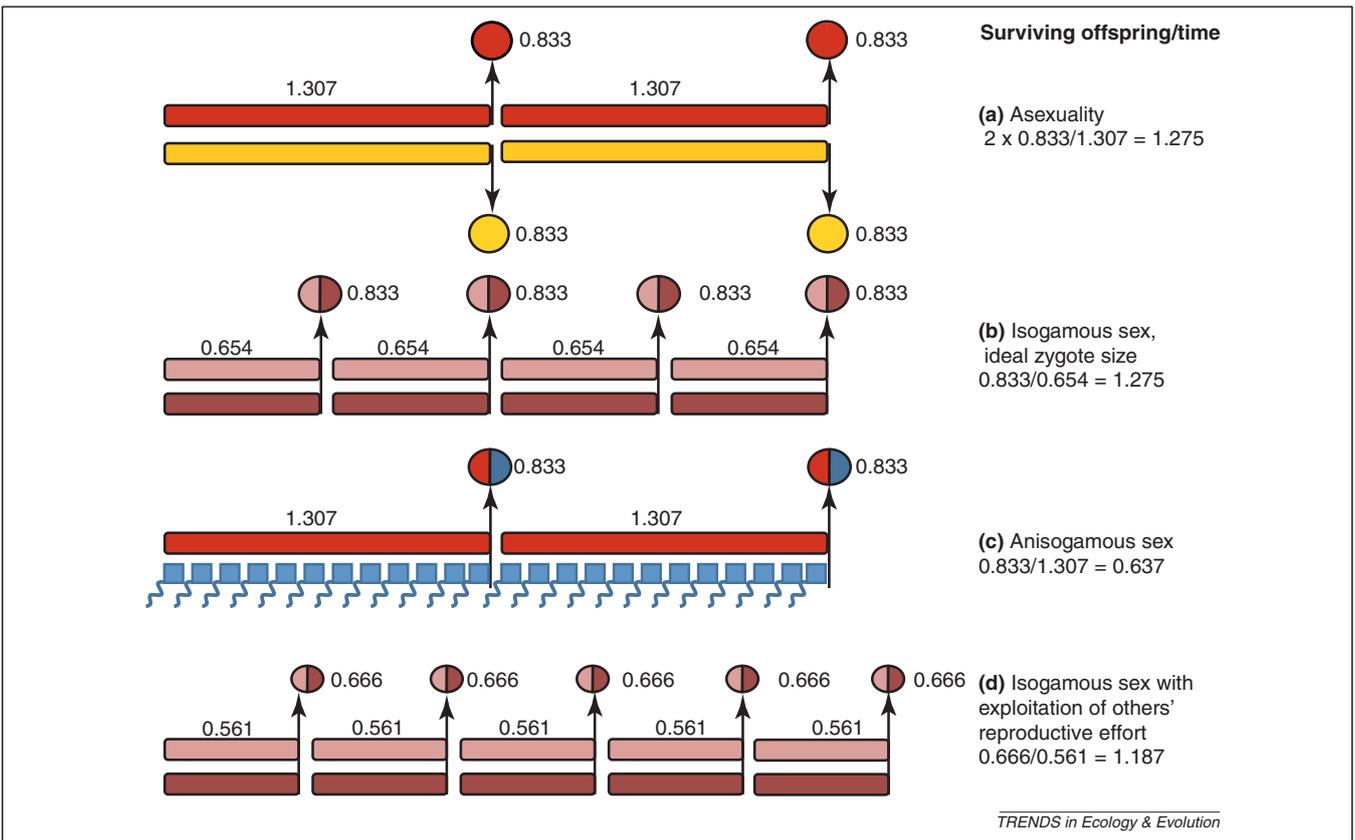


Figure 1. Comparison of reproductive output under different types of reproduction (a–d). For illustrative purposes, each population comprises two individuals. They gather resources as time flows from left to right. As soon as they gather enough resources (horizontal bar) to form a gamete or offspring, it is produced (vertical arrow). Gametes are fertilised to form a zygote (sphere). The colour of the sphere indicates the gametic contributions of the parent(s). Larger gametes require more resources. Here, resource acquisition is assumed to be constant over time so larger gametes take longer to produce. The precise numerical examples for the size–number trade-off follow case C in Matsuda & Abrams [9]. The numbers along the resource acquisition bars refer to gamete size (minimally small for sperm; for clarity, only a few sperm are drawn) or zygote size (for asexuals). The number alongside the zygote refers to its predicted survival [= size⁶/(1 + size⁶)]. The total reproductive output is shown on the right, expressed as (survival of zygote)/(time taken to produce a zygote).

systems, which correlates with greater levels of polyandry. When selection on male sexual competitiveness decreases female reproductive output [24], polyandry can elevate the cost of sex beyond twofold. Males now hinder female

reproduction instead of merely playing an inefficient zero-sum paternity game [25].

What matters is not maleness *per se*, but how two parents contribute resources to offspring. Parenting

involves conflict and cooperation, reflected in upwards and downwards deviations from a twofold cost. The economics of parental investment can even generate conflict over provisioning offspring in isogamous organisms that obviously never experience male–female sexual conflict [9,26]. Parental conflict over gamete size under isogamy is conceptually no different from male–female conflict over care: each parent benefits if the other invests more [27,28]. Sex involves no cost if parents produce gametes that are half the size of an asexually produced zygote (Figure 1a versus 1b), but a cost reappears if both parents are selected to ‘work less’ for each offspring than the other and instead invest in new mating opportunities. With isogamy, conflict-driven gamete size reduction leads to smaller zygotes than those of an asexual parent (Figure 1a versus 1d). The consequent lower overall growth rate of an isogamous lineage resembles a male-imposed cost, in that its source is inefficient resource use under conflict. The difference is that neither of the two mating types can be identified as the sole culprit.

Numbers of individuals matter too

There are at least two other ways in which the cost of male production might deviate from twofold. First, sexual lineages do not always invest 50% of their resources into sons. For example, local mate competition favours female-biased offspring sex ratios, so the cost of male production becomes less than twofold. The growth rate of a sexual lineage depends on how many daughters are produced and how much parental investment they receive relative to sons.

Second, interactions between males and females are not restricted to mating and caring for offspring. Males sometimes influence the access of females to resources used in offspring production. For example, if males are the larger sex, they can deplete local resources faster than can females, making resources unavailable for female reproduction. This cost is lowered if males are shorter lived (female-biased adult sex ratio) or if males and females use different resources. Of course, if females are driven to utilise poorer resources by male behavioural dominance, then differential use of resources is an additional symptom of a cost, rather than a mitigating factor [29,30].

The unavoidable costs of searching for and choosing mates

Sex requires that compatible gametes, or mates, encounter each other, even when males and females do not exist (isogamy). Although failure to find a mate is predominantly considered a male problem (not influencing female reproductive output, hence disqualified as a cost of sex), there is indirect and direct evidence for a cost of sex owing to mate-finding difficulties. If insects are excluded, approximately one-third of animal species are hermaphrodites [31]. Hermaphroditism is even more widespread in plants. The difficulty of finding mates is widely implicated in the evolution of hermaphroditism [32], so its widespread occurrence suggests that sexual organisms pay significant costs to locate mates. In species with distinct sexes, such as insects (which are never hermaphroditic), matelessness can create breeding failures for a significant proportion of females (e.g. [33]).

Sexual organisms can evolve traits to deal with low mate availability. Females can avoid reproductive delays or breeding failure by investing in mate location, but such investment probably trades off with fecundity. Mate finding can also increase the risk of predation or disease transmission, again reducing female fecundity. Diseases can also be sexually transmitted during copulation. Asexual reproduction avoids all these costs.

Female mate choice for genetic benefits, even though often selected for relative to mating indiscriminately [34], can still decrease the reproductive output of sexual females relative to asexuality. Choosiness reduces the availability of suitable mates, aggravating the aforementioned costs (except, perhaps, when choosy females avoid diseased mates or identify more helpful males). Any decline in the relevant fitness components again qualifies as a cost of sex; in this case, not directly imposed by males but ‘self-imposed’ by females to produce more attractive sons. Female sexual traits can also become genetically correlated with traits expressed and selected for in males [35], so that female reproductive output declines.

The mechanics of meiosis are costly for some

Although researchers often concentrate on sex in large, multicellular eukaryotes, it arose in unicellular eukaryotes [36]. Sex imposes unique costs in these tiny organisms. Most unicellular organisms are isogamous [37] so male production costs do not exist. Given that isogamy, despite conflict (Figure 1d), uses resources more efficiently than when males interfere with resource use (Figure 1a,b), one might expect sex to be especially prevalent in unicellular eukaryotes. However, many species engage in sex infrequently, or forgo it completely [38]. Why?

In unicellular eukaryotes, asexual reproduction via apomixis involves mitosis followed by cell division. Mitosis can take as little as 15 minutes. By contrast, sex involves meiosis, which usually takes more than 10 hours (e.g. [7]), and is generally estimated to take 5 to 100 times longer than mitosis for unicellular organisms [4,38]. Sexual organisms are selected to reduce the time needed for meiosis, but fundamental mechanical constraints appear to exist (e.g. orientation, movement and correct pairing of chromosomes [4,38]). This time cost of sex is, however, potentially irrelevant when asexual reproduction involves asexuality via automixis, as this also involves meiosis (Box 1).

To what extent meiosis lowers the rate of reproduction depends on the extent of pre-reproductive development. The time cost is trivial in species that reach reproductive maturity slowly or exhibit extended delays between reproductive bouts. This could be one reason why sex is more prevalent in larger, multicellular organisms than in unicellular ones, even though the former often pay significant costs of male production. Meiosis and gamete production can occur simultaneously with growth, sexual maturation and other preparations for breeding in multicellular organisms, whereas this is impossible for unicellular organisms.

No-one escapes the cost of recombination except some asexuals

Sex enhances the potential for evolution because recombination expands the range of gene combinations exposed to

selection [2,3,5,14,39–42]. The ability to parcel together beneficial genes or to separate them from harmful mutants is among the key long-term benefits of sex. In the short term, however, recombination is costly because it breaks up successful gene combinations [3,4].

The success of particular gene combinations relies on non-additive genetic interaction. Consider a locus for which heterozygote fitness exceeds that of either homozygote. Asexuality preserves heterozygosity in offspring if reproduction involves apomixis, whereas heterozygosity declines at least 50% per sexual generation [4]. Similar issues apply across loci. Whether non-additive interactions arise through dominance or epistasis, recombination can break up successful gene combinations faster than it creates new successful genotypes.

The reshuffling of sets of genes that worked well together in the previous generation reflects the cost of sex owing to recombination: new genotypes in the next generation are, on average, less fit [2–5,7,43]. Formally, the decline in the mean genotypic value (fitness) of a trait owing to recombination is referred to as ‘genetic slippage’ [44,45]. This is arguably the most general cost of sex because epistatic interactions are very common. It does not require anisogamy, sexual conflict or a life history for which the time cost of meiosis is significant. It does, however, depend on how selection fluctuates across space and time. The more stable the environment, the greater the likelihood that reshuffled genotypes will be less fit than previously successful ones, increasing the cost of recombination.

All this assumes apomictic parthenogenesis, but what about automixis? Automixis involves recombination, even if genes are only reshuffled within one diploid individual. Automixis leads to loss of heterozygosity, possibly resulting in homozygosity across the entire genome after a few generations [20]. Any heterozygote advantage is lost, and deleterious recessive alleles can cause significant problems. However, if a homozygous genotype is well adapted to the environment, this will be preserved.

Measuring the cost of sex: so much trouble in the real world

Measuring the cost of sex involves at least three challenges. First, calculating the costs of sex is more complicated than measuring the net fitness difference between asexual and sexual females. Positive effects of sex should not be doubly accounted for as both ‘benefits’ and ‘reduced costs’.

The second issue relates to male production. This cost depends on the economics of offspring production with or without males. However, should one compare the performance of asexual and sexual females coexisting in a single population? Or should one compare the growth rate of separate (discrete) asexual and sexual populations? Does the phrase ‘without males’ mean that males are not used as mates and neither are sons produced, or that no males exist in the vicinity? The distinction matters whenever males can potentially affect the reproductive output of asexual females. For example, if males deplete food resources, then both sexual and asexual females experience a male-induced reduction in food availability.

The appropriate comparison depends on the question asked. To determine how the cost of sex affects the likelihood

that an asexual mutant will invade a sexual population, one must investigate populations where asexuals are exposed to potential male-imposed costs. It might, simultaneously, also be necessary to compare discrete sexual and asexual populations if asexual populations can become established and avoid male-imposed costs. Both comparisons are needed to predict the dynamics in spatial mosaics where some habitat patches only contain asexuals, whose offspring then disperse to compete elsewhere with sexuals (for a study performing both comparisons, see Wolinska and Lively [46]). It is important to be explicit about the comparison being made when measuring the cost of sex.

Third, over what timescale should the costs of sex be measured? An inappropriate scale creates problems for empirical and theoretical studies alike. For example, recent theoretical work claims that the growth rate of asexuals is far less than twice that of sexuals if recruitment only occurs when adults die [8], implying a smaller cost of male production in long-lived ‘K-selected’ than in short-lived ‘r-selected’ organisms. This approach conflates population growth rate differences over a short time interval (during which neither asexual nor sexual adults have had time to die) with a more important consideration: how large should the benefits of sex be (e.g. higher survival of sexual offspring) to counter the cost of reduced production of daughters. This remains twofold regardless of the position along the r–K continuum posited in [8].

Nonetheless, it is potentially relevant that the absolute speed with which asexuals can invade sexual populations will depend on the rates at which existing adults die [8]. It influences the temporal scale over which selection occurs. If parasites evolve much faster than their hosts, the likelihood that offspring experience a different selective environment from their parents is elevated. Recombination then becomes less costly, and possibly even offers a short-term benefit to sex [47].

Real-world examples of measuring the cost of sex

Despite difficulties in quantifying the effects of sex in ecologically relevant settings [44], several empirical studies have estimated how sex affects various fitness components. Others have investigated key theoretical statements (e.g. whether the cost is twofold) by testing the implicit ‘all else is equal’ assumption for sexual and asexual females. The logic is that if reproductive output or mortality do not differ then, given investment of resources into males, sex must be costly. Several studies have found that asexuals have higher mortality [48–50] or lower fecundity [51–53], but the basis of such differences is often poorly understood. Much research has focused on genetic effects improving some fitness components of sexuals (e.g. less well-adapted asexual genotypes), which shifts the focus to the benefits of sex. Given the complicated nature of inefficiencies related to male production, it would be worthwhile to direct more research effort towards male and female resource use before, during and after parental investment.

Jokela *et al.* [54] tested whether ‘all else is equal’ [3] in the snail *Potamopyrgus antipodarum* by comparing clonal and sexual life-history traits. No significant differences were found in the field, predicting a significant cost of

males. Indeed, in laboratory conditions, the clonal population rapidly outgrew the sexual population. This was not quantified on a generational timescale, leaving it uncertain whether the difference was twofold.

Most *Daphnia* species reproduce by cyclical parthenogenesis (CP). Asexuality prevails in benign conditions, whereas sex produces diapausing eggs that survive harsh conditions [55]. Many CP organisms also have obligately parthenogenetic variants and these full asexuals have been used to measure the cost of males and of recombination. Such comparisons are, however, complicated by ecological differences between sexual and asexual reproduction, which appear to be necessary for their stable coexistence [56]. This makes interpreting the comparison as one between sexual and asexual reproduction by otherwise equivalent females problematic.

In *Daphnia pulex*, the asexual variant still produces males, but at a far lower proportion of its offspring than the sexual form. Male production costs are larger for sexuals, yet the number of daughters produced did not differ between asexual and sexual females, because asexuals were less fecund [57]. In the same species, a cost of males under non-competitive conditions did not prevent sexuals from outperforming asexuals in a setting with direct competition [46]. Again, of course, to equate the costs of sex with net fitness differences remains problematic as this assumes no short-term benefit of sex.

Another CP cladoceran, *Daphnia pulicaria*, has been used to investigate recombination costs. Both the costs and the benefits of sex were correlated with the frequency of sex [44]. Here, the short-term cost of sex was equated with the mean change in genotypic values of traits such as adult size and clutch size (a quantification of genetic slippage), and benefits were equated with the variance (suggesting long-term adaptive potential).

The impression that empirical studies often use very different definitions of a 'cost of sex' is strengthened by complications described in a recent study of the rotifer *Brachionus calyciflorus* [58]. Asexuals outcompete sexual females in the short term as they produce neither males nor diapausing eggs (neither contributes to immediate population growth). Yet wholly asexual lineages would die out during a prolonged period of habitat deterioration that only diapausing eggs survive. There can be no cost of sex when asexual fitness is zero (as no fitness component of a sexual female can be lower). The immediate per-generation costs must necessarily fluctuate over time. Explicit dynamic calculations of competing schedules of asexual and sexual reproduction are a better way to understand this situation than any simplistic attempt to compare 'benefits' and 'costs'.

Clearly, in the real world, the costs of sex are less clear-cut than theory suggests. The cost of males is theoretically straightforward if males and females play simple reproductive roles. Studies of *D. pulex* [46,57] show, however, that the cost can be masked by changes in other fitness components, making it difficult to detect it when comparing net fitness. This is unsurprising. The very organisms that allow researchers to compare asexual and sexual forms must, for whatever reasons, exhibit modest differences in net fitness between the two types for both to persist. In effect, the dice are loaded when picking relevant study species.

Focusing on the cost of males is further complicated in cladoceran and rotifer species when asexual strains still produce males. Theoretically, an intermediate investment in males by an asexual strain can be optimal [18,19], so that both the costs of male production and of genome dilution come into play (Box 3). Frustratingly, species that readily lend themselves to measurements of costs are often those that do not fit neatly in either the cost of males or cost of genome dilution category. Future work would benefit from considering both costs [18,19].

Finally, there is a conceptual issue: when testing for a cost of sex, should one state how well an asexual would outcompete sexuals once its asexual life cycle is perfected, or how asexuality performs in real life where, in multicellular eukaryotes, it is a derived trait [59]? Recently evolved asexuals often exhibit vestiges of sexual reproduction [20]. If they are maladaptive in an asexual context, phylogenetic inertia can protect sexuals against invasion by asexuals.

Conclusion: the costs of sex depend on who you are, and what you become

Most work on sex focuses on identifying advantages that counter theoretically assumed costs. Can this approach be justified? Although the cost of males is rarely exactly twofold, it is probably still the major cost of sex for most multicellular eukaryotes with males and females. Assuming twofoldness is, nevertheless, dangerous in specific contexts. It implicitly assumes that sex involves males, that males and females have highly divergent reproductive roles but otherwise similar life histories, and that there are no other costs to sex.

Thinking carefully is especially important when considering the origins of sex. Anisogamy is almost certainly a derived evolutionary state [3,37], so the cost paid by the first isogamous sexual organisms was far less than twofold. The initial evolutionary origins of sex in unicellular eukaryotes probably involved an almost cost-free transition from the state in Figure 1a to that in Figure 1b and then, because of parental conflict, to that in Figure 1d (although they would also have paid other types of cost). Once anisogamy evolved, the costs of sex became more diverse, changing markedly depending on male and female behaviour. This is ultimately a reminder that the ease with which a system can return to asexuality will be influenced by numerous additional constraints imposed by vestiges of sexual life (reviewed in [20]).

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