The ovotestis: an underdeveloped organ of evolution

Angus Davison

Summary

In animals that have separate sexes (gonochorists), many sperm are produced to fertilise a few eggs. As the male germline undergoes more mitoses, so the accumulated mutation frequency is elevated in sperm compared with ova, and evolution is 'male-driven'. In contrast, in many hermaphroditic animals, a single organ—the ovotestis—produces both ova and sperm. Since self-renewing cells in the ovotestis may give rise to both cell types throughout life, ova in hermaphrodites could in theory have undergone as many cell divisions as sperm. Here, I consider some possible effects of the ovotestis on evolution. In particular, I hypothesise that the accumulated mutation frequency of nuclear genes in hermaphrodites (including species that change sex) may reach twice that compared with gonochorists. There may be an even greater increase in the mitochondrial mutation frequency. Further developmental studies and the accumulation of comparative data should allow hypothesis testing. If the prediction is correct, then it may provide the most-straightforward explanation for the extraordinary diversity of mitochondrial DNA in some hermaphrodites, especially molluscs. *BioEssays* **28:642–650, 2006. © 2006 Wiley Periodicals, Inc.**

Introduction

Ever since Haldane's prediction that rates of mutation should be elevated in males[1,2] (mostly male) scientists have searched for evidence that evolution is 'male-driven'. Although it took around 65 years,[3–6] it is now beyond doubt that the accumulated mutation frequency in some male germlines is elevated compared with females.[4,7,8] The debate now tends to be more concerned with the magnitude of the effect (represented by α, the ratio of mutations originating in males compared to females[7]), and whether the causes of variation between estimates are real or due to ascertainment bias.[4,7,9–12]

The basic rationale behind the argument for male-driven evolution is simple. In species that have separate sexes (gonochorists), there is differential investment in reproduction, since many small, inexpensive sperm attempt to fertilise few, large and costly eggs, a condition known as anisogamy. For

male mammals, spermatogonia are self-renewing, so undergo mitosis throughout the reproductive life cycle whereas, in female mammals, the total number of oogonia is fixed at birth. As the male germline must in general undergo more mitoses, the accumulated mutation frequency should be elevated in sperm compared with ova, so males contribute a greater part towards the overall rate of evolution compared with females.[1,2] The longer a male lives and reproduces, then the greater the accumulated mutation frequency and the greater the value of α. Male-driven evolution may therefore only be of relevance in animals that are long-lived—in *Drosophila*, the ratio of germline cell divisions changes from a weak female bias to a weak male bias as the reproductive age increases;[13] there is no difference in accumulated mutation frequency between the sexes.[13,14]

If male-driven evolution is real and not artefactual, then one condition is that the accumulated mutation frequency is proportional to the rate of evolution, or the rate at which mutations are fixed. Most of these mutations will be neutral or nearly neutral. There is also an implicit assumption that, while a compensatory repair mechanism could in theory evolve to counteract a high male mutation rate, it does not always do so, otherwise male-driven evolution would not have been detected in mammals,[4] birds[5,6] and fish.[3] Finally, "male-driven evolution" is often assumed to refer to differences that are generated during mitosis rather than meiosis. This is not always the case, however, since several human sequences evolve more rapidly in males than females as a consequence of meiotic events rather than the number of germline mitoses.[7]

Strangely, there does not seem to be an explicit statement in the literature as to the expectation for male-driven evolution in hermaphrodites. One study on hermaphrodite plants states that it was "remarkable" to find evidence for male-driven evolution in plants, because the germ and somatic cells are shared for much of life[15]. Instead the main concern in previous reviews has been to discuss the theory, issues surrounding the estimation of α, and explain contradictory results.[7–10,16] In part, the bias in the literature towards gonochorists and male-driven evolution must result from the fact that we have viewed species predominantly as having separate sexes. The reality is somewhat different, however. Twenty of 28 animal phyla have at least some hermaphroditic species and around ten are almost exclusively hermaphroditic:

Annelida, Bryozoa, Chaetognatha, Cnidaria, Ctenophora, Entoprocta, Gastrotricha, Mollusca, Platyhelmithes, and Porifera[17,18] (Table 1). In some phyla, hermaphroditism is ubiquitous whereas, in others, it is rarer or confined to specific groups. Although I do not consider them further here, most plants are also hermaphroditic.

An elevated rate of evolution in hermaphrodites

So, perhaps the question is better put as "why shouldn't male-driven evolution occur in hermaphrodites?" (Box 1). Although it does not seem to have been generally appreciated by those that study molecular evolution, in many hermaphrodites, a single organ—the ovotestis—produces both eggs and sperm (Table 1). If self-renewing germ cells in the ovotestis continually differentiate into both sperm and eggs, then both eggs and sperm should have undergone approximately the same number of cell divisions. The expectation therefore is that the accumulated mutation frequency in male and female germline stem cells should be about equal. Male-driven evolution should not occur in hermaphrodites. Moreover, since ova in the ovotestis of long-lived species will undergo more mitoses than in the equivalent gonochorist, then the accumulated mutation frequency in a hermaphrodite will be on average higher than the corresponding gonochorist—providing that the same conditions apply as described above (e.g. a high mutation rate is not counteracted by selection for modifiers that reduce it; α increases with increasing life-span).

If it is assumed that, in an ovotestis, the accumulated mutation frequency in ova is as high as in sperm, and if the theoretical ratio between the accumulated mutation frequency in hermaphrodites and gonochorists is h, then as male-driven evolution, α, tends to increase in gonochorists, h asymptotically approaches 2 in the equivalent hermaphrodite (Fig. 1). This can be expressed as:

$$h \text{ (biparentally inherited genome)} = \frac{\text{hermaphrodite } (a^m + a^f)}{\text{gonochorist } (a^m + a^f)} \quad (1)$$

where a^m = accumulated mutation frequency in the male germ line, a^f = accumulated mutation frequency in the female germ line. If twice as much evolution occurs in the male genome of a gonochorist ($\alpha = 2$), then the rate of evolution in the equivalent

Table 1. The occurrence of hermaphroditism and sex-change in the Metazoa. Hermaphrodites either have an ovotestis, are digonic, or male and female gametes develop in a loose aggregation. Metazoan phyla that lack hermaphrodites are not shown. See main text for literature sources

	Frequency of hermaphroditism	**Gonadal development**	**Occurrence of sex-change**
Basal Lineages			
Cnidaria	ubiquitous in Anthozoa absent otherwise	loose	protandrous/protogynous sex change; protogynous hermaphroditism
Ctenophora	ubiquitous	digonic	absent
Porifera	ubiquitous	loose[a]	protandrous/protogynous sex change both common
Lophotrochozoa			
Annelida	ubiquitous	digonic	protandrous/protogynous sex change; protandrous/protogynous hermaphroditism
Bryozoa	ubiquitous	digonic	many species protandrous hermaphrodites
Entoprocta	common, perhaps ubiquitous	ovotestis	protandrous sex change common
Gnathostomulida	ubiquitous	digonic	protandrous sex change
Mollusca	ubiquitous; except Cephalopoda where absent	ovotestis	protandrous hermaphroditism common; protandrous sex change rarer, but common in bivalves and prosobranchs
Phoronida	present	ovotestis	
Platyhelminthes	ubiquitous	digonic	protandrous sex change rare
Ecdysozoa			
Arthropoda	rare in crustacean; absent or very rare otherwise	ovotestis[b] digonic[c]	protandrous sex change and protandrous hermaphroditism in crustaceans; protogynous sex change rare
Gastrotricha	ubiquitous	digonic	protandrous/protogynous sex change
Nematoda	present	ovotestis	
Tardigrada	parachelate heterotardigrades	ovotestis	
Deuterostomes			
Chaetognatha	ubiquitous	digonic	
Echinodermata	(almost) absent	digonic	protandrous sex change
Urochordata	ubiquitous	ovotestis/digonic	limited protandrous/protogynous hermaphroditism
Vertebrata	common in fish; rare or absent otherwise	ovotestis	protandrous sex change common; protogynous sex change less so

[a]Sponges are said to lack gonads; instead germ cells are scattered throughout the mesohyl.
[b]In Phyllopoda and Decapoda.
[c]In Remipedia and Cirripedia.

hermaphrodite could be 33% higher [$h = (2+2)/(2+1) = 1.33$]; if $\alpha = 5$, then $h = 1.67$ [$h = (5+5)/(5+1)$]. The rate of evolution will also contribute directly to the standing variation within a population since neutral molecular variation (π) is theoretically dependent upon only the mutation rate μ and the effective population size N_e.[19]

More significantly, there may be a much greater amplification of the rate of evolution in maternally inherited cytoplasmic organelles, such as mitochondria. The explanation is that, in gonochorists, cytoplasmic organelles are usually inherited clonally,[20] via the mother, so undergo relatively few cell divisions (there are, however some exceptions[21]). Although, in hermaphroditic animals, they are usually also inherited maternally, the female germline will have undergone as many (or almost as many) cell divisions as the male. In the absence of any counter-evolutionary DNA repair mechanisms, then the accumulated mutation frequency should be correspondingly high. The expectation is that the relationship between α and h should be linear (Fig. 1).

$$h \text{ (maternally inherited genome)} = \frac{\text{hermaphrodite } (a^f)}{\text{gonochorist } (a^f)} \quad (2)$$

If $\alpha = 5$ in a gonochorist, then $h = 5/1$ for maternally inherited organelles.

There is one further consideration: in hermaphrodites, the effective population size of organelles is in theory twice that of normal (all else being equal), since all individuals in a population can potentially contribute to the next generation. Under equivalent circumstances as described above ($\alpha = 2$ or 5), the expectation therefore is that the neutral variation of organelles should be 4 or 10 times as great in a hermaphrodite compared with an equivalent gonochorist (by "equivalent" I mean a gonochoristic species that does not differ substantially from a closely related hermaphroditic species, except for the defining difference in their reproduction).

Together, these theoretical calculations give rise to several hypotheses for the patterns expected if female germ cells
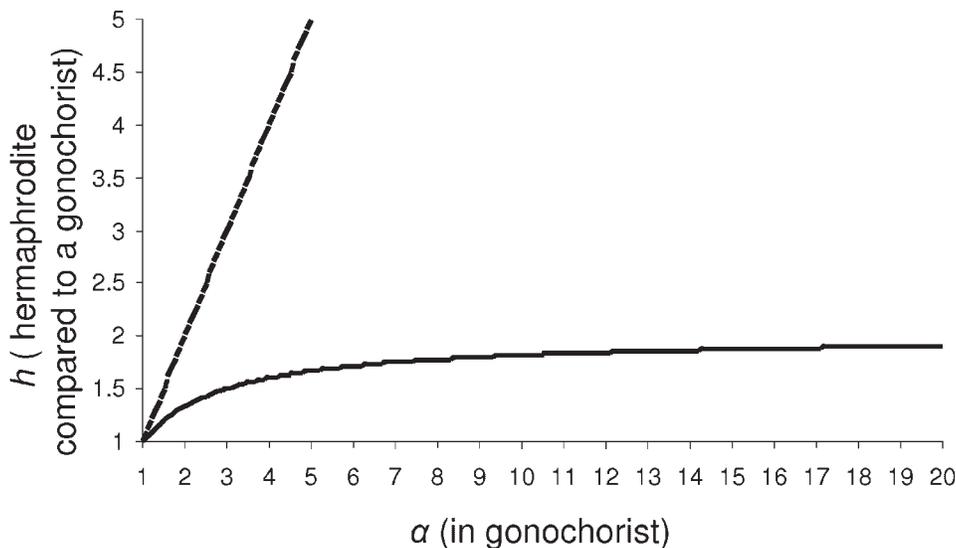

Figure 1. Effects of male biased mutation in a gonochorist relative to a hermaphrodite, when all else is equal. Theoretical relationship between α, the male: female bias in the accumulated mutation frequency in a gonochorist, and h, the ratio between the accumulated mutation frequency in a hermaphrodite and a gonochorist. By definition, there is no male-driven evolution in hermaphrodites (α is set to 1) Maternally and biparentally inherited genomes are indicated by a dotted or solid line, respectively. In theory, if the rate of evolution and α are known in a gonochorist, then the relationship between α and h could be used to calculate a corrective factor for the molecular clock in a hermaphrodite.

Box 2

(1) In hermaphrodites, the mutation rate in ova may approach that in sperm, unlike in gonochorists. **(2)** Since ova in an ovotestis potentially undergo almost as many mitoses as sperm, then the rate of evolution of nuclear genes in hermaphrodites may reach twice that compared with gonochorists. **(3)** There will be a much greater amplification of the relative mutation rate in maternally-inherited cytoplasmic organelles. **(4)** Providing that both kinds of germ cell develop from a common, self-renewing stem cell, those species that change sex from male to female (protandrous sex change) or male to hermaphrodite (protandrous hermaphroditism) may have a greater rate of molecular evolution than those that change from female to male (protogynous sex change) or female to hermaphrodite (protogynous hermaphroditism).

As with male-driven evolution in gonochorists, the above predictions are also dependent upon much the same assumptions: the accumulated mutation frequency should be proportional to the rate of evolution; a lack of a compensatory repair mechanism in response to a higher mutation rate; most mutation taking place during mitosis rather than meiosis.

undergo almost as many cell divisions as male germ cells in hermaphrodites (Box 2). As the potential evolutionary significance of what might be termed the "ovotestis effect" does not seem to have been realised previously, I investigated the main groups in which it might be a significant factor.

Germ cell development in hermaphrodites

About half of all hermaphrodite animal phyla contain species that have an ovotestis (Table 1); the other hermaphrodite animal groups are digonic (Box 1). I needed further precise information on ovotestis development to determine absolutely whether male and female germ cells develop from a common progenitor or stem cell. Unfortunately, except for some model organisms, hermaphrodites are a neglected group, so detailed histological and developmental information is lacking for most.[22,23] Moreover, for several organisms, the later stages of the developmental path of the male and female germ cells have been determined but the precise origins of the cells remain unknown. A complete review is therefore not yet possible.

Nematodes

Undoubtedly, the most-detailed information on germ cell development in hermaphrodites comes from studies of the nematode *Caenorhabitis elegans*.[22] In hermaphroditic *C. elegans*, the germline is organised in a linear fashion: the most distal cells in the ovotestis undergo mitosis, those in the middle enter meiosis and proximal cells differentiate to sperm or ova. The mitotic region of the germline thus renews the supply of mitotic cells, as well as supplying meiotic cells for oogenesis or spermatogenesis; whether a particular cell becomes a sperm or ovum is dependent upon an interaction between several key genes.[24–26] Over a lifetime, each worm generates about 2000 germ cells, of which more than 1000 undergo apoptosis, so that around 300 sperm and a slightly larger number of oocytes finally mature.[27] Although there have been few comparative studies on nematodes, the mode of germ cell development in *C. elegans* (and sex determination?) may be common, as a similar path has also been found in a distantly related species, *Pristionchis pacificus*.[28]

Fish

Perhaps the next-best-studied group are fish.[29,30] Many fish are hermaphrodites and able to change sex, usually from female to male, but occasionally from male to female.[31] In simultaneously hermaphroditic fish species, the ovotestis is divided into separate male and female regions,[30,32,33] so it is reasonable to suppose (though not proven) that different sets of primordial germ cells differentiate into sperm or oocytes (the same is true of ascidians[34,35]). In sequentially sex-changing fish, however, adult sex change involves the degeneration of functional gonadal tissue of the first sex, then the growth and maturation of the gonadal tissue of the opposite sex.[32,36–40] Presently, in most if not all species, it is unknown whether the proliferating tissue of the second sex differentiates at the same time as the first sex, then becomes dormant, or else whether it differentiates at a later stage from a common and self-renewing primordial germ cell. In at least some cases, the latter may be correct: in *Epinephelus microdon*, sex change takes place within ex-ovarian lamellae, as bipotential primordial germ cells begin to differentiate.[40]

There are several other salient features in fish. First, gonadal maturation does not begin until rather late in development. Even in gonochoristic species, such as zebrafish, it is not possible to determine sex until after several weeks of development.[41] Second, it has been argued that the 'default' fish sex is female, because, even in protandrous species, development of male cells is both preceded and followed by ovarian development[32] (the first set of female gametes never mature, and are absorbed, so can easily be missed). Thus, in some gonochoristic species, the developing gonads differentiate into ovaries in the female and, much later, into testes in the male. In zebrafish, gonads first develop into ovary-like gonads.[41] In half of the individuals, the ovary-like gonads become ovaries and produce oocytes. In the other half, they develop into testes and the fish become male.

Molluscs

In hermaphroditic molluscs, the consensus is that, in most species, both types of gamete originate from indeterminate germ cells in every part of the ovotestis, and that this occurs throughout the life cycle.[42] Gametes arise by proliferation of germinal cells that line the follicle wall. In species where reproduction is seasonal, the ovotestis degenerates at the end of each year, then regenerates again during spring.[42] Unfortunately, it is presently unknown whether ova and sperm arise from the same self-renewing germ cells during ordinary development, or over the seasonal cycles.

Many molluscs are also long-lived—land snails live for five or more years, so go through several reproductive seasons; some clams live for up to 150 years and produce large quantities of sperm, which is linked evolutionarily to multiple mating and fierce sperm competition.[17] Both of these factors could also increase the number of germline mitoses.[7,12] Many molluscs also have a period in which they are male before becoming hermaphrodite, or else change sex entirely.[31,43] In addition, just as in fish, gonadal differentiation (and thus sex determination in gonochoristic species) does not occur until rather late in the development of many molluscs. In gonochoristic marine bivalves, in particular, sex is not determined until the germ cells are differentiated, whether in the larva or in the juvenile.[42] The cell types present in the testis, ovary or ovotestis are apparently identical until a late stage of development.

Other groups

In the remaining groups, even less is known about development. In hermaphroditic tardigrades such as *Macrobiotus* and *Amphibolus*, mature gametes of both types are never separated by physical barriers, and there are at least two cycles of egg production.[44,45] In some groups of crustaceans, specifically the phyllopods, decapods and isopods, both protandrous hermaphroditism and sex change are relatively common, with protogynous hermaphroditism/sex change less so.[31,46] In the clam shrimp *Eulimnadia*, the anterior portion of the hermaphrodite ovotestis is ovarian tissue and the smaller posteror portion is testis.[47] Thus, while in this species and other branchiopods,[48] there is an apparent functional separation, it remains unclear whether the separation occurs early or late in development. In the tadpole shrimp *Triops*, the separation is less—male germ cells originate along both the longitudinal tubules and the lateral branches at whose tips female germ cells differentiate[49]—so it is conceivable that both germ cell types derive from the same stem cells.[49] Finally, while most hermaphrodite echinoderms have separate ovaries and testes,[18] in those that change sex the male secondary structures degenerate very rapidly with increasing size, sperm production ceases and the gonad becomes a developing ovary filled with large yolk-filled oocytes. Transitional gonads with previtellogenic oocytes and mature

spermatozoa have been observed, suggesting that sex change could be initiated from a common germ cell.[50]

What then are the expectations for molecular evolution and diversity in the above-mentioned hermaphrodites? There are several clear predictions (Box 3). Since both male and female germ cells undergo around the same number of cell divisions in *C. elegans*, then the rate of evolution through the male and female germline should be about equal (providing that the potential and unquantified contribution of males is ignored). However, the overall rate of evolution will be relatively low (including mitochondria), since the number of cell divisions to generate egg and sperm are rather few: hermaphrodite nematodes produce few sperm, a probable consequence of lowered reproductive effort due to a high degree of selfing and lessened sperm competition (the effects of inbreeding on evolution have been reviewed elsewhere[51−53]).

In most *simultaneously* hermaphroditic fish, the expectation is arguably for unequal rates of evolution, because the oocytes and sperm are produced within different regions of the ovotestis. In *sequentially* hermaphroditic fish, however, it seems more likely that male and female germ cells develop from a common self-renewing germline stem cell. If so, then equal and high rates of molecular evolution are expected, with particularly high rates of mitochondrial evolution, especially in the few species that are protandrous and sex-changing.

As for the other animal groups, the expectations are much the same as in fish. The main difference is that, unlike in fish where protogyny is most common, in most invertebrates protandry is more common. It is therefore even more likely that some long-lived invertebrates may have an accelerated rate of molecular evolution.

Box 3
Some general predictions for the impact of the ovotestis effect. **(1)** Male-driven evolution in gonochorists; male-driven evolution absent or much reduced in ovotestis-bearing hermaphrodites. **(2)** Male-driven evolution reduced in gonochorists with late-developing gonads. **(3)** Elevated rate of molecular evolution of nuclear genes in ovotestis-bearing hermaphrodites. **(4)** A highly elevated rate of evolution of maternally-inherited organelles (e.g. mitochondria); no effect on the evolution of paternally-inherited factors. **(5)** More rapid molecular evolution in protandrous species compared with protogynous species; simultaneous hermaphrodites intermediate. **(6)** Lower rate of evolution and reduced male-driven evolution in self-fertilizing hermaphrodites. **(7)** Paternal inheritance of organelles more common in some hermaphrodites.

Analysis of molluscs and the ovotestis

It has been noted frequently that molluscs may have exceptionally high rates of molecular evolution, especially of mitochondrial DNA,[54−59] but probably also at some nuclear loci.[60−64] Within species, mitochondrial divergence in many land snails is of the order of 5 to 30%, depending upon the species and gene fragment used. In *Mandarina* land snails, the rate of evolution has been estimated to be 10% per million years at a gene that is normally slowly evolving.[55] Strikingly, it has also been found that two species of protandrously hermaphroditic littorinid snails have a remarkably elevated rate of evolution compared with their gonochoristic relatives.[65,66] There are no other significant differences between the species, except for their hermaphroditism.[66]

Several authors have previously tried to explain the high molecular diversity in land snails.[54,62] The main explanations have been (1) unusually structured or exceptionally large populations, (2) ancient isolation of populations that consequently diverged, (3) selection acting to generate and maintain variability, and (4) an exceptionally high mutation rate.[54] There is some evidence that population structure helps promote or maintain genetic diversity with molluscs,[67] but it is doubtful that it is the whole explanation. I am not aware of any satisfactory data on (2) or (3), except the special case of doubly uniparental inheritance[68,69] (see below). A high mutation rate (4) is by itself an unsatisfactory explanation, because it begs the question of why the mutation rate should be so high? The ovotestis effect could therefore provide the most-straightforward explanation for the high genetic diversity and high rates of molecular evolution in molluscs.

Doubly uniparental inheritance in bivalve molluscs

Species in three families of bivalves are exceptional because the sexes contain two separate types of mitochondrial DNA (F and M), which are transmitted through the female and male line, hence the term doubly uniparental inheritance (DUI).[68,69] Since F and M genomes are transmitted uniparentally, evolve rapidly and do not usually recombine, they are markedly divergent. Mussels also have a characteristic sex ratio bias—individual females tend to give birth to offspring that are (1) mostly one sex or the other, (2) about equal proportions of male and female, or (3) hermaphrodite.[70,71]

One explanation for the rapid evolution of both M and F mitochondria that has not been considered is the ovotestis effect. F mitochondria are in cells that could undergo almost as many cell divisions as the M types, because the gonad—a functional ovotestis—does not differentiate into an ovary or testis until late in development, when the germline stem cells have already proliferated (the M genome evolves more rapidly than the F, due to a relaxation in selective constraint,[72] and/or because there are still more mitotic divisions in differentiated males).

In addition, DUI may have originally evolved in a simultaneously hermaphroditic species, or else one in which sex is determined late and by the development of the gonad. The reasoning is that, if mitochondrial DNA is always inherited maternally, and males are heterogametic, then mitochondria can never evolve a function that directly benefits the male, unless the same function simultaneously benefits the female.[73,74] Therefore, with paternal inheritance of mitochondrial DNA in a species with separate sexes, it is not in the interests of maternally expressed sex-linked genes to "allow" paternal inheritance to persist, since sex-linked genes and maternally inherited mitochondria should be co-adapted. In contrast, in simultaneous hermaphrodites, especially those with an ovotestis, no such conflicts exist. Instead, the nuclear genome will tend to be associated with the mitochondrial genome that gives the greatest inclusive fitness, irrespective of whether it is inherited maternally or paternally.

As selection should be weak against paternally inherited mitochondria in hermaphrodites, they could be retained for some time in the ovotestis, just as occurs in mussels.[75] However, for paternally inherited mitochondria to persist in the long term, then they must ensure their own transmission. One way would be to masculinise the ovotestis (as is believed to occur[70,71,76]), so that paternal transmission and sex changing would become associated together.

Hypothesis testing

Except for encouraging further studies on the development of gonadal stem cells, especially in the ovotestis, the approach to determining whether the ovotestis effect is real or not must depend upon the comparative method.[77−80] If mutation is equal in both types of germ cell in a hermaphrodite, then one consequence is that the latter should evolve more rapidly at a molecular level. The molecular evolution of mitochondrial DNA will be even faster compared with nuclear sequences. Protandrous species may evolve more rapidly than protogynous species.

From a purely practical perspective, it would be unwise to use a gonochorist-calibrated molecular clock in a hermaphrodite lineage, at least not without a corrective factor. Fortunately, most authors already use and interpret the data from molecular clocks with caution, and are increasingly using a 'local' clock method,[81] where possible. All of the usual precautions must be extra-rigorously applied when dealing with hermaphrodites.

Does the ovotestis effect contribute to long-term molecular evolution?

While I have hypothesised as to the potential evolutionary effects of the ovotestis in hermaphrodite organisms, some of the underlying assumptions may not apply, or be relevant, in all circumstances. The ovotestis effect is only appropriate when all else is equal between comparable gonochorists and

hermaphrodites, species that have similar life histories and levels of sexual selection.

The longevity of the organism, in particular, may be a key factor in determining whether male-driven evolution is important in both hermaphrodites and gonochorists. Generation time is also a confounding factor.[82] In *Drosophila*, male-driven evolution is lacking;[13] the best explanation is that, because individuals are short-lived, male germline stem cells undergo around the same number of mitoses as female stem cells. There is therefore no expectation that short-lived hermaphrodites should have an elevated rate of mutation compared with their equivalent short-lived, gonochorist relatives. In terms of long-term molecular evolution, other factors such as effective population size and generation time may be much more important.

Another consideration is that mutation rate is likely to evolve. The ovotestis effect might not be relevant since a high mutation rate may lead to selection for modifiers that reduce the mutation rate. On the one hand, the physiological cost of reducing mutation may be the most important factor in setting the genomic mutation rate, regardless of the benefits of mutation in producing new adaptive variation.[83] On the other hand, as the vast majority of mutations that have a phenotypic effect are likely to be individually deleterious, then this may maintain persistent selection in favour of lower mutation rates.[83] Most likely, both factors have a role in maintaining low rates of mutation. Presently, the high rate of mitochondrial evolution is thought to reflect high rates of DNA damage, coupled with more limited DNA repair;[84] it is also clear that the mutation rate of mitochondrial DNA can evolve, at least in plants.[85,86]

Finally, the degree of sexual selection may be important. If hermaphrodites self-fertilise more frequently than they outcross (e.g. *C. elegans*), then selection should lead them to reduce their investment in the male function.[53] If there are fewer sperm, then there should be fewer mitoses and fewer mutations. With a high degree of selfing, then the expectation is that male-driven evolution should be lacking. There could even be slight bias towards female-driven evolution.

Conclusion

Although considerable effort is and has been devoted to estimating and understanding male-driven evolution, there have been few investigations of the corollary, a rapid though equal male- and female-driven evolution. Moreover, the evolutionary significance of having a single ovotestis, compared with the 'normal' situation, has rarely been considered. As the bias is due to an overemphasis of the research effort on a few, model gonochorists, then this may lessen as more genome sequences accumulate from diverse groups, including that of the first molluscs. Direct evidence for a heightened rate of evolution in hermaphrodites is currently lacking, so I would like to encourage research that complements the

valuable work that has already been done. It remains to be determined whether the ovotestis effect is general, or may only apply under limited circumstances.

References

1. Haldane JBS. 1947. The mutation rate of the gene for haemophilia, and its segregation ratios in males and females. Ann Eugenic 13:262–271.
2. Haldane JBS. 1935. The rate of spontaneous mutation of a human gene. J Genet 31:317–326.
3. Ellegren H, Fridolfsson AK. 2003. Sex-specific mutation rates in salmonid fish. J Mol Evol 56:458–463.
4. Shimmin LC, Chang BHJ, Li WH. 1993. Male driven evolution of DNA sequences. Nature 362:745–747.
5. Ellegren H, Fridolfsson AK. 1997. Male-driven evolution of DNA sequences in birds. Nat Genet 17:182–184.
6. Axelsson E, Smith NGC, Sundstrom H, Berlin S, Ellegren H. 2004. Male-biased mutation rate and divergence in autosomal, Z-linked and W-linked introns of chicken and turkey. Mol Biol Evol 21:1538–1547.
7. Hurst LD, Ellegren H. 1998. Sex biases in the mutation rate. Trends Genet 14:446–452.
8. Ellegren H. 2002. Human mutation—blame (mostly) men. Nat Genet 31:9.
9. Hurst LD, Ellegren H. 2002. Mystery of the mutagenic male. Nature 420:365–366.
10. Li WH, Yi SJ, Makova K. 2002. Male-driven evolution. Curr Opin Genet Dev 12:650–656.
11. Bohossian HB, Skaletsky H, Page DC. 2000. Unexpectedly similar rates of nucleotide substitution found in male and female hominids. Nature 406:622–625.
12. Bartosch-Harlid A, Berlin S, Smith NGC, Moller AP, Ellegren H. 2003. Life history and the male mutation bias. Evolution 57:2398–2406.
13. Bauer VL, Aquadro CF. 1997. Rates of DNA sequence evolution are not sex-biased in Drosophila melanogaster and D. simulans. Mol Biol Evol 14:1252–1257.
14. Betancourt AJ, Presgraves DC, Swanson WJ. 2002. A test for faster X evolution in Drosophila. Mol Biol Evol 19:1816–1819.
15. Whittle CA, Johnston MO. 2002. Male-driven evolution of mitochondrial and chloroplastidial DNA sequences in plants. Mol Biol Evol 19:938–949.
16. Kirkpatrick M, Hall DW. 2004. Male-biased mutation, sex linkage, and the rate of adaptive evolution. Evolution 58:437–440.
17. Michiels NK. 1998. Mating conflicts and sperm competition in simultaneous hermaphrodites. In: Birkhead T, Møller AP, editors. Sperm competition and sexual selection. London: Academic Press. p 219–254.
18. Ruppert EE, Fox RS, Barnes RD. 2004. Invertebrate Zoology. Belmont, USA: Thomson Brooks/Cole.
19. Graur D, Li WH. 1999. Fundamentals of Molecular Evolution. Sunderland, MA: Sinauer.
20. Birky CW. 2001. The inheritance of genes in mitochondria and chloroplasts: Laws, mechanisms, and models. Annu Rev Genet 35:125–148.
21. Barr CM, Neiman M, Taylor DR. 2005. Inheritance and recombination of mitochondrial genomes in plants, fungi and animals. New Phytol 168:39–50.
22. Lee DL. 2002. The Biology of Nematodes. London and New York: Taylor & Francis.
23. Extavour CG, Akam M. 2003. Mechanisms of germ cell specification across the metazoans: epigenesis and preformation. Development 130:5869–5884.

24. Eckmann CR, Crittenden SL, Suh N, Kimble J. 2004. GLD-3 and control of the mitosis/meiosis decision in the germline of Caenorhabditis elegans. Genetics 168:147–160.

25. Crittenden SL, Eckmann CR, Wang LT, Bernstein DS, Wickens M, et al. 2003. Regulation of the mitosis/meiosis decision in the Caenorhabditis elegans germline. Philos Trans R Soc Lond Ser B-Biol Sci 358:1359–1362.

26. Crittenden SL, Bernstein DS, Bachorik JL, Thompson BE, Gallegos M, et al. 2002. A conserved RNA-binding protein controls germline stem cells in Caenorhabditis elegans. Nature 417:660–663.

27. Gumienny TL, Lambie E, Hartwieg E, Horvitz HR, Hengartner MO. 1999. Genetic control of programmed cell death in the Caenorhabditis elegans hermaphrodite germline. Development 126:1011–1022.

28. Rudel D, Riebesell M, Sommer RJ. 2005. Gonadogenesis in Pristionchus pacificus and organ evolution: development, adult morphology and cell-cell interactions in the hermaphrodite gonad. Dev Biol 277:200–221.

29. Braat AK, Speksnijder JE, Zivkovic D. 1999. Germ line development in fishes. Int J Dev Biol 43:745–760.

30. Hoar WS, Randall DJ. 1969. Fish Physiology. New York: Academic Press.

31. Allsop DJ, West SA. 2004. Sex-ratio evolution in sex changing animals. Evolution 58:1019–1027.

32. Shapiro DY. 1992. Plasticity of gonadal development and protandry in fishes. J Exp Zool 261:194–203.

33. Lee YH, Du JL, Yueh WS, Lin BY, Huang JD, et al. 2001. Sex change in the protandrous black porgy, Acanthopagrus schlegeli: A review in gonadal development, estradiol, estrogen receptor, aromatase activity and gonadotropin. J Exp Zool 290:715–726.

34. Okada T, Yamamoto M. 1999. Differentiation of the gonad rudiment into ovary and testis in the solitary ascidian, Ciona intestinalis. Dev Growth Differ 41:759–768.

35. Takamura K, Fujimura M, Yamaguchi Y. 2002. Primordial germ cells originate from the endodermal strand cells in the ascidian Ciona intestinalis. Dev Genes Evol 212:11–18.

36. Schartl M. 2004. A comparative view on sex determination in medaka. Mech Dev 121:639–645.

37. Frisch A. 2004. Sex-change and gonadal steroids in sequentially-hermaphroditic teleost fish. Rev Fish Biol Fish 14:481–499.

38. Garcia-Diaz MM, Lorente MJ, Gonzalez JA, Tuset VM. 2002. Morphology of the ovotestis of Serranus atricauda (Teleostei, Serranidae). Aquat Sci 64:87–96.

39. Bruslesicard S, Reinboth R, Fourcault B. 1994. Germinal potentialities during sexual state changes in a protandric hermaphrodite, Amphiprion frenatus (Teleostei, Pomacentridae). J Fish Biol 45:597–611.

40. Bruslesicard S, Debas L, Fourcault B, Fuchs J. 1992. Ultrastructural study of sex inversion in a protogynous hermaphrodite, Epinephelus microdon (Teleostei, Serranidae). Reprod Nutr Dev 32:393–406.

41. Uchida D, Yamashita M, Kitano T, Iguchi T. 2002. Oocyte apoptosis during the transition from ovary-like tissue to testes during sex differentiation of juvenile zebrafish. J Exp Biol 205:711–718.

42. Tompa AS, Verdonk NH, van den Biggelaar JAM. 1984. The Mollusca: Volume 7 Reproduction. Orlando, Florida: Academic Press. 486 p.

43. Wright WG. 1988. Sex change in the Mollusca. Trends Ecol Evol 3:137–140.

44. Rebecchi L, Guidi A, Bertolani R. 2000. Maturative pattern of the ovotestis in two hermaphrodite species of eutardigrades. Invertebr Reprod Dev 37:25–34.

45. Bertolani R, Rebecchi L. 1999. Tardigrada. In: Knobil E, Neill JD, editors. Encyclopedia of Reproduction. Volume 4 Pro-Z: Academic Press.

46. Brook HJ, Rawlings TA, Davies RW. 1994. Protogynous sex change in the intertidal isopod Gnorimosphaeroma oregonense (Crustacea, Isopoda). Biol Bull 187:99–111.

47. Zucker N, Cunningham M, Adams HP. 1997. Anatomical evidence for androdioecy in the clam shrimp Eulimnadia texana. Hydrobiologia 359:171–175.

48. Tommasini S, Sabelli FS. 1992. Morphological and functional aspects of the female gonad of the conchostracan Leptestheria dahalacensis Ruppel, 1837 (Crustacea, Branchiopoda), and a comparison with the gonads of other Branchiopoda. Can J Zool 70:511–517.

49. Scanabissi F, Mondini C. 2002. A survey of the reproductive biology in Italian branchiopods—Part B. The male gonad of Lepidurus apus lubbocki Brauer, 1873 (Notostraca). Hydrobiologia 486:273–278.

50. Sewell MA. 1994. Small size, brooding, and protandry in the apodid sea cucumber Leptosynapta clarki. Biol Bull 187:112–123.

51. Charlesworth D. 2003. Effects of inbreeding on the genetic diversity of populations. Philos Trans R Soc Lond Ser B-Biol Sci 358:1051–1070.

52. Charlesworth D, Wright SI. 2001. Breeding systems and genome evolution. Curr Opin Genet Dev 11:685–690.

53. Charlesworth D, Charlesworth B. 1981. Allocation of resources to male and female functions in hermaphrodites. Biol J Linn Soc 15:57–74.

54. Thomaz D, Guiller A, Clarke B. 1996. Extreme divergence of mitochondrial DNA within species of pulmonate land snails. Proc R Soc Lond B Biol Sci 263:363–368.

55. Chiba S. 1999. Accelerated evolution of land snails Mandarina in the oceanic Bonin Islands: Evidence from mitochondrial DNA sequences. Evolution 53:460–471.

56. Dillon RT, Frankis RC. 2004. High levels of mitochondrial DNA sequence divergence in isolated populations of freshwater snails of the genus Goniobasis Lea, 1862. Am Malacol Bull 19:69–77.

57. Quattro JM, Chase MR, Rex MA, Greig TW, Etter RJ. 2001. Extreme mitochondrial DNA divergence within populations of the deep-sea gastropod Frigidoalvania brychia. Mar Biol 139:1107–1113.

58. Goodacre SL, Wade CM. 2001. Patterns of genetic variation in Pacific island land snails: the distribution of cytochrome b lineages among Society Island Partula. Biol J Linn Soc 73:131–138.

59. Pinceel J, Jordaens K, Backeljau T. 2005. Extreme mtDNA divergences in a terrestrial slug (Gastropoda, Pulmonata, Arionidae): accelerated evolution, allopatric divergence and secondary contact. J Evol Biol 18:1264–1280.

60. Davison A. 1999. Isolation and characterization of long compound microsatellite repeat loci in the land snail, Cepaea nemoralis L (Mollusca, Gastropoda, Pulmonata). Mol Ecol 8:1760–1761.

61. Davison A, Chiba S, Kawata M. 2004. Characterization of 17 microsatellite loci in the Japanese land snail genera Mandarina, Ainohelix, and Euhadra (Mollusca, Gastropoda, Pulmonata). Mol Ecol Notes 4:423–425.

62. Davison A. 2002. Land snails as a model to understand the role of history and selection in the origins of biodiversity. Popul Ecol 44:129–136.

63. Guiller A, Arnaud JF, Vautrin D, Solignac M. 2000. Highly polymorphic microsatellite markers in the landsnail Helix aspersa (Mollusca Gastropoda). Mol Ecol 9:1191–1193.

64. Wade CM, Mordan PB, Clarke B. 2001. A phylogeny of the land snails (Gastropoda : Pulmonata). Proc R Soc Lond B Biol Sci 268:413–422.

65. Williams ST, Reid DG, Littlewood DTJ. 2003. A molecular phylogeny of the Littorininae (Gastropoda : Littorinidae): unequal evolutionary rates, morphological parallelism, and biogeography of the Southern Ocean. Mol Phylogenet Evol 28:60–86.

66. Reid DG. 1986. Mainwaringia nevill, 1885, a littorinid genus from Asiatic mangrove forests, and a case of protandrous hermaphroditism. J Molluscan Stud 52:225–242.

67. Foltz DW. 2003. Invertebrate species with nonpelagic larvae have elevated levels of nonsynonymous substitutions and reduced nucleotide diversities. J Mol Evol 57:607–612.

68. Skibinski DOF, Gallagher C, Beynon CM. 1994. Mitochondrial DNA inheritance. Nature 368:817–818.

69. Zouros E, Ball AO, Saavedra C, Freeman KR. 1994. An unusual type of mitochondrial DNA inheritance in the blue mussel Mytilus. Proc Natl Acad Sci USA 91:7463–7467.

70. Saavedra C, Reyero MI, Zouros E. 1997. Male-dependent doubly uniparental inheritance of mitochondrial DNA and female-dependent sex-ratio in the mussel Mytilus galloprovincialis. Genetics 145:1073–1082.

71. Kenchington E, MacDonald B, Cao LQ, Tsagkarakis D, Zouros E. 2002. Genetics of mother-dependent sex ratio in blue mussels (Mytilus spp.) and implications for doubly uniparental inheritance of mitochondrial DNA. Genetics 161:1579–1588.

72. Stewart DT, Kenchington ER, Singh RK, Zouros E. 1996. Degree of selective constraint as an explanation of the different rates of evolution of gender-specific mitochondrial DNA lineages in the mussel Mytilus. Genetics 143:1349–1357.

73. Randerson JP, Hurst LD. 1999. Small sperm, uniparental inheritance and selfish cytoplasmic elements: a comparison of two models. J Evol Biol 12:1110–1124.

74. Zeh JA, Zeh DW. 2005. Maternal inheritance, sexual conflict and the maladapted male. Trends Genet 21:281–286.

75. Cao LQ, Kenchington E, Zouros E. 2004. Differential segregation patterns of sperm mitochondria in embryos of the blue mussel (Mytilus edulis). Genetics 166:883–894.

76. Zouros E. 2000. The exceptional mitochondrial DNA system of the mussel family Mytilidae. Genes Genet Syst 75:313–318.

77. Felsenstein J. 1985. Phylogenies and the comparative method. Am Nat 125:1–15.

78. Bromham L. 2002. Molecular clocks in reptiles: Life history influences rate of molecular evolution. Mol Biol Evol 19:302–309.

79. Bromham L, Rambaut A, Harvey PH. 1996. Determinants of rate variation in mammalian DNA sequence evolution. J Mol Evol 43:610–621.

80. Harvey PH, Pagel M. 1991. The comparative method in evolutionary biology. Oxford: Oxford University Press.

81. Kishino H, Thorne JL, Bruno WJ. 2001. Performance of a divergence time estimation method under a probabilistic model of rate evolution. Mol Biol Evol 18:352–361.

82. Martin AP, Palumbi SR. 1993. Body size, metabolic rate, generation time, and the molecular clock. Proc Natl Acad Sci U S A 90:4087–4091.

83. Sniegowski PD, Gerrish PJ, Johnson T, Shaver A. 2000. The evolution of mutation rates: separating causes from consequences. BioEssays 22: 1057–1066.

84. Martin AP. 1995. Metabolic rate and directional nucleotide substitution in animal mitochondrial DNA. Mol Biol Evol 12:1124–1131.

85. Cho Y, Mower JP, Qiu YL, Palmer JD. 2004. Mitochondrial substitution rates are extraordinarily elevated and variable in a genus of flowering plants. Proc Natl Acad Sci USA 101:17741–17746.

86. Parkinson CL, Mower JP, Qiu YL, Shirk AJ, Song KM, et al. 2005. Multiple major increases and decreases in mitochondrial substitution rates in the plant family Geraniaceae. BMC Evol Biol 5:73.