A comparative test of a theory for the evolution of anisogamy

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Why are sperm small and eggs large? The dominant explanation for the evolution of gamete size dimorphism envisages two opposing selection pressures acting on gamete size: small gametes are favoured because many can be produced, whereas large gametes contribute to a large zygote with consequently increased survival chances. This model predicts disruptive selection on gamete size (i.e. selection for anisogamy) if increases in zygote size confer disproportional increases in fitness (at least over part of its size range). It therefore predicts that increases in adult size should be accompanied by stronger selection for anisogamy. Using data from the green algal order Volvocales, we provide the first phylogenetically controlled test of the model's predictions using a published phylogeny and a new phylogeny derived by a different method. The predictions that larger organisms should (i) have a greater degree of gamete dimorphism and (ii) have larger eggs are broadly upheld. However, the results are highly sensitive to the phylogeny and the mode of analysis used.

Keywords: anisogamy; uniparental inheritance; conflict hypothesis; gamete size; Volvocales; Volvocaceae

1. INTRODUCTION

Gametes come in two varieties in many organisms. In some cases they are a different size (i.e. sperm and eggs), but in others the gametes are the same size (i.e. isogametes) but are differentiated into two mating types (plus and minus or α and α). Assuming isogamy is the ancestral condition we can ask why did the gametes change in relative size? A number of theories have been presented (Parker *et al.* 1972; Cosmides & Tooby 1981; Hoekstra 1982; Cox & Sethian 1985; Dusenbery 2000) (for a review see Hoekstra 1987). Here we test the dominant explanation for the evolution of gamete dimorphism (Parker *et al.* 1972; Maynard Smith 1978; Bulmer 1994).

Parker, Baker and Smith's (Parker *et al.* 1972) model (henceforth the PBS model) for the evolution of gamete dimorphism proposes two opposing selection pressures acting on gamete production: the number of gametes produced and the fitness of the resulting zygote (which is assumed to be some function of its size). Gamete number and gamete contribution to zygote fitness necessarily trade off against each other.

A male produces multiple sperm and can therefore fertilize numerous eggs whilst contributing little to the zygote. In this regard, males parasitize the investment in eggs provided by females. However, for disruptive selection on gamete size to occur the PBS model requires that, at least over part of its size range, zygote fitness must increase disproportionately with volume (i.e. an increase in zygote size must confer a more than proportional increase in its fitness). It is suggested that this is more likely in multicellular organisms than in unicellular organisms (Parker *et al.* 1972). In the former, the transition from zygote to complex multicellular organism requires more provisioning, so an increase in zygote size is likely to confer a significant benefit. Conversely, increased provisioning on the part of a unicellular organism need

not lead to a significant increase in zygote fitness (Parker *et al.* 1972; Bell 1985). The PBS model therefore predicts that, as adult size increases, so does selection for anisogamy (Parker *et al.* 1972; Maynard Smith 1978). One should therefore observe a greater degree of anisogamy in larger species.

A further prediction concerns the relationship between egg size and adult size. If a larger zygote brings about a disproportionate gain in fitness in larger species then egg size should also increase with adult size (Bell 1985). Clearly, increased egg size might lead to an increase in the degree of anisogamy; the two could form part of the same prediction. However, this is not necessarily the case. Increased anisogamy could come about by a decrease in sperm size with egg size staying the same.

On the other hand, the PBS model predicts that the degree of gamete dimorphism will be greatest in organisms with the largest zygotes. Therefore, as organisms become larger, not only should their zygotes increase in size, but also a greater proportion of zygote volume should come from the egg. Thus, an increase in egg size with adult size is a strong prediction of the model.

The green algae have been the traditional testing ground for the PBS model (Knowlton 1974; Madsen & Waller 1983; Bell 1985). Members of the group exhibit variation in both size and gamete dimorphism (e.g. *Chlamydomonas reinhardtii* is isogamous and unicellular, whilst the oogamous *Volvox rousetii* has up to 50 000 cells per colony) (see the references in electronic Appendix B available on The Royal Society Web site). Tests of the PBS model's prediction that the degree of anisogamy should increase with adult size have yielded equivocal results. While Knowlton (1974) and Bell (1985) suggested that the pattern may be found in the Volvocales, data accumulated by Madsen & Waller (1983) on pond-living algae indicated numerous exceptions (see also Bell 1978). Furthermore, in agreement with the PBS model, Bell (1985) reported that zygote size increases with adult size in the Volvocales. However, interpretation of all these

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Figure 1. Contrasts in the anisogamy ratio versus contrasts in $\log_{10}(\text{protoplasmic volume})$ using the Coleman (1999) phylogeny (Spearman's $\rho = -0.002$, $n = 31$ and $p > 0.05$).

results is impossible as in no case was phylogenetic non-independence taken into account. The new phylogeny (Coleman 1999) of the Volvocales now makes the appropriate test possible using the method of independent contrasts (Purvis & Rambaut 1995). Furthermore, we derived our own phylogeny in order to investigate how sensitive the results of this test are to the particular tree used.

2. METHODS

(a) *The existing phylogeny*

The phylogeny (see electronic Appendix A available on The Royal Society's Web site) is an amalgamation of the two cladograms published by Coleman (1999) using variations in the two internal transcribed spacers (ITS-1 and ITS-2) flanking the 5.8S ribosomal gene. This phylogeny will subsequently be referred to as the Coleman (1999) phylogeny. One of Coleman's

(1999) cladograms was derived using a parsimony method. Given the potential unreliability of this method of tree reconstruction, we derived an alternative phylogeny in order to test the robustness of our comparative tests to the particular tree used.

(b) *Our phylogeny*

The taxa and GenBank accession numbers used were a subset of those in Coleman's (1999) study. Where that phylogeny supported a monophyletic clade consisting entirely of different isolates of one species, we used a representative member of that species. However, if a species was predicted to be paraphyletic, more than one isolate was used in the analysis. The original phylogeny (Coleman 1999) reported GenBank entry U67020 as being *Pandorina morum* (UTEX871). It is in fact a *Volvox dissipatrix* isolate that appears elsewhere in the phylogeny. We only included this once in our analysis.

The phylogeny contains 44 taxa representing 38 different species. The species represented by more than one different isolate are as follows: *Pleodorina indica* (two isolates), *Eudorina illinoisensis* (two isolates), *P. morum* (four isolates) and *V. dissipatrix* (two isolates). The DNA sequence for alignment was a region encompassing the two internal transcribed spacers (ITS-1 and ITS-2) flanking the 5.8s ribosomal gene. The largest of these sequences was 1273 nucleotides long. The sequences were aligned using CLUSTALX (available at <http://www.hgmp.mrc.ac.uk/Registered/Menu/alphabet.html>). The reason for the length disparity between our sequences and Coleman's (1999) sequences is that we included the RNA gene whereas she did not.

A distance matrix was calculated using TREE-PUZZLE (formerly known as Puzzle). This programme uses quartet puzzling, a maximum-likelihood technique that reconstructs all possible groups of four taxa (quartets) that can be formed from n sequences. These quartet trees then serve as starting points for reconstructing a set of optimal n -taxon trees (Strimmer & Von Haeseler 1996). The distance matrix computed by TREE-PUZZLE was fed into Weighbor (Bruno *et al.* 2000). This is a

Table 1. *Summary of results using both phylogenies and the raw data alone*

test	raw data (no phylogeny)	Coleman (1999) phylogeny	Weighbor phylogeny
anisogamy ratio versus $\log_{10}(\text{protoplasmic volume})$	$F = 41.2$, d.f. = 30, $p < 0.001$ and $r^2 = 58.7$ Spearman's $\rho = 0.77$ and $p < 0.005$	$F = 23.4$, d.f. = 31, $p < 0.001$ and $r^2 = 41.9$ Spearman's $\rho = -0.002$ and n.s.	$F = 65.9$, d.f. = 36, $p < 0.001$ and $r^2 = 64.3$ Spearman's $\rho = 0.53$ and $p = 0.005$
$\log_{10}(\text{anisogamy ratio})$ versus $\log_{10}(\text{protoplasmic volume})$	$F = 35.5$, d.f. = 30, $p < 0.001$ and $r^2 = 55.0$ Spearman's $\rho = 0.74$ and $p < 0.005$	$F = 0.23$, d.f. = 31, $p = 0.64$ and $r^2 = 0$ Spearman's $\rho = -0.038$ and n.s.	$F = 13.7$, d.f. = 36, $p = 0.001$ and $r^2 = 26.1$ Spearman's $\rho = 0.47$ and $p = 0.005$
$\log_{10}(\text{egg or isogamete volume})$ versus $\log_{10}(\text{protoplasmic volume})$	$F = 29.9$, d.f. = 25, $p < 0.001$ and $r^2 = 55.5$ Spearman's $\rho = 0.75$ and $p < 0.005$	$F = 13.9$, d.f. = 24, $p = 0.001$ and $r^2 = 34.9$ Spearman's $\rho = 0.29$ and n.s.	$F = 9.2$, d.f. = 27, $p = 0.005$ and $r^2 = 23.3$ Spearman's $\rho = 0.44$ and $p < 0.02$
sperm number versus egg number	$F = 49.3$, d.f. = 17, $p < 0.001$ and $r^2 = 75.5$ Spearman's $\rho = 0.87$ and $p < 0.005$	$F = 19.7$, d.f. = 16, $p < 0.001$ and $r^2 = 53.9$ Spearman's $\rho = 0.65$ and $p < 0.005$	$F = 17.9$, d.f. = 19, $p = 0.001$ and $r^2 = 47.1$ Spearman's $\rho = 0.68$ and $p < 0.005$
egg number versus $\log_{10}(\text{protoplasmic volume})$	$F = 47.4$, d.f. = 25, $p < 0.001$ and $r^2 = 66.4$ Spearman's $\rho = 0.82$ and $p < 0.005$	$F = 52.1$, d.f. = 26, $p < 0.001$ and $r^2 = 66.3$ Spearman's $\rho = 0.43$ and $p < 0.02$	$F = 33.0$, d.f. = 30, $p < 0.001$ and $r^2 = 51.6$ Spearman's $\rho = 0.49$ and $p < 0.005$

Figure 2. Contrasts in the anisogamy ratio versus contrasts in $\log_{10}(\text{protoplasmic volume})$ using the Weighor phylogeny (Spearman's $\rho = 0.47$, $n = 37$ and $p < 0.005$). The regression analysis was highly significant ($F = 65.9$, d.f. = 36, $p < 0.001$ and $r^2 = 64.3$). Furthermore, the intercept was not significantly different from zero ($p = 0.28$).

weighted neighbour-joining method of tree construction that produces quantitatively and qualitatively similar results to maximum-likelihood methods but is much faster. Furthermore, it does not suffer from the problem of long-branch attraction inherent in standard neighbour-joining methods. We used the HKY model of substitution with gamma-distributed rates.

Our phylogeny (henceforth referred to as the Weighor phylogeny) (see electronic Appendix A) is similar to that reported by Coleman (1999). In many cases groups of species are preserved in a similar topology to the original phylogeny, but the relationships between these blocks are different. Many of the conclusions that Coleman (1999) drew from her phylogeny are also supported here. For example, we also see a split between the four-celled *Gonium* species and those with more than four cells. Furthermore, we also find an association between *Volvox* species and *P. morum*. Most strikingly, our phylogeny also reinforces the notion that *Volvox* is a paraphyletic genus.

(c) The tests and the data

The PBS hypothesis predicts a positive correlation between contrasts in the degree of anisogamy and contrasts in the amount of provisioning that goes into adult growth (Parker *et al.* 1972). *A priori*, one would expect protoplasmic volume (i.e. volume of an individual protoplast $\times$ number of cells) to reflect this degree of provisioning most accurately. Using 'colony cell number' as such a measure is vulnerable to variations in cell size and 'colony volume' would include the space enclosed by hollow spherical colonies and so does not truly represent provisioning. Subsequently, we therefore used protoplasmic volume as our measure of provisioning in the adult. Most cells that were not spherical were prolate spheroids (i.e. a spindle-shaped ellipsoid). If a , b and c are the radii of the three axes, then their volume equals $4/3\pi abc$.

The upper limit of the range was used in the analyses when different literature sources disagreed on the value of a parameter for a particular species. This is the most appropriate measure as it ensures that we are considering mature colonies and not juveniles.

The anisogamy ratio is defined as macrogamete volume divided by microgamete volume. For cases in which this

Figure 3. Contrasts in $\log_{10}(\text{egg volume or isogamete volume})$ versus contrasts in $\log_{10}(\text{protoplasmic volume})$ using the Coleman (1999) phylogeny (Spearman's $\rho = 0.29$, $n = 24$ and $p > 0.05$).

information was not available (particularly in species with sperm packets), the number of sperm in a packet was used as the anisogamy ratio. This assumes that the investment in a sperm packet and in an egg is approximately equal. Hence, if there are 32 sperm in a packet then each one is $1/32$ the volume of an egg. While this is not ideal, given that sperm packets appear to be approximately the same size as eggs, we do not believe that this approximation adds a large degree of error.

Both of the model's predictions were tested using the raw data alone and the method of independent contrasts (Purvis & Rambaut 1995) using both phylogenies. We noted that one large contrast in the Coleman (1999) phylogeny had a disproportionate affect on the results (e.g. figure 1). We therefore based our conclusions on non-parametric statistics applied to the data, although the equivalent parametric statistics are reported in table 1.

3. RESULTS

(a) Does anisogamy increase with adult size?

The raw data (not taking phylogenetic history into account) support the PBS model's prediction that $\log_{10}(\text{female gamete volume/male gamete volume})$ should increase with $\log_{10}(\text{protoplasmic volume})$. The two are very significantly correlated (Spearman's $\rho = 0.77$ and $p < 0.005$) (table 1).

However, when using the Coleman (1999) phylogeny, we found no significant correlation between contrasts in $\log_{10}(\text{female gamete volume/male gamete volume})$ and contrasts in $\log_{10}(\text{protoplasmic volume})$ (Spearman's $\rho = -0.002$, $n = 31$ and $p > 0.05$) (table 1 and figure 1). Furthermore, this result was not affected by the decision to log the anisogamy ratio before calculating contrasts. There was no significant correlation between the unlogged ratio and $\log_{10}(\text{protoplasmic volume})$ (Spearman's $\rho = -0.038$, $n = 31$ and $p > 0.05$) (table 1).

However, this result was highly sensitive to the particular phylogeny used in the analysis. When we used the Weighor phylogeny there was a very significant correlation between contrasts in the anisogamy ratio and contrasts in $\log_{10}(\text{protoplasmic volume})$ (Spearman's $\rho = 0.47$, $n = 37$ and $p < 0.005$) (table 1 and figure 2). Furthermore, this

Figure 4. Contrasts in $\log_{10}$ (egg volume or isogamete volume) versus contrasts in $\log_{10}$ (protoplasmic volume) using the Weighor phylogeny (Spearman's $s = 0.44$, $n = 28$ and $p < 0.02$). The regression analysis was highly significant ($F = 9.2$, d.f. = 27, $p < 0.005$ and $r^2 = 23.3$). Furthermore, the intercept was not significantly different from zero ($p = 0.93$).

was not sensitive to the decision to log the anisogamy ratio (Spearman's $s = 0.53$, $n = 37$ and $p < 0.005$) (table 1).

(b) Does egg size increase with adult size?

A further prediction of the PBS model is that zygote size (and, hence, egg size) should increase with adult size. As previously reported by Bell (1985), the raw data support this prediction: $\log_{10}$ (protoplasmic volume) and $\log_{10}$ (egg or isogamete volume) are significantly correlated (Spearman's $s = 0.75$ and $p < 0.005$) (table 1).

However, this result changed when the group's phylogenetic history was taken into account. There was no significant correlation between $\log_{10}$ (egg or isogamete volume) and $\log_{10}$ (protoplasmic volume) when using the Coleman (1999) phylogeny (Spearman's $s = 0.29$, $n = 24$ and $p > 0.05$) (table 1 and figure 3). This result contradicts the prediction of the PBS model. On the other hand, there was a significant correlation between contrasts in egg size and contrasts in $\log_{10}$ (protoplasmic volume) when we used the Weighor phylogeny (Spearman's $s = 0.44$, $n = 28$ and $p < 0.02$) (table 1 and figure 4).

One possible reason for the discrepancy between the two results is that the Coleman (1999) phylogeny may have reconstructed ancestral nodes incorrectly. Certainly, one would expect both phylogenetic methods to reconstruct the relationships between terminal taxa most accurately.

A different test uses contrasts between these terminal taxa only and, thus, does not rely on correct ancestral node reconstruction. We used this method in order to ascertain what proportion of terminal closest relative species pairs supported the PBS model's hypothesis prediction with respect to egg size. Support was taken to be cases in which both adult size and egg size increased or both decreased.

Where the phylogeny suggested that two species were unambiguously each other's closest relative (e.g. *Astrephomene gubernaculifera* and *Astrephomene perforata*), they were used in the analysis. In other cases, where one species could have multiple others as its closest relative

(e.g. *Volvox obversus* with *Volvox carteri f. nagariensis* and *Volvox carteri f. kawasakiensis*), the second taxon making up the contrast was chosen at random. This analysis yielded 13 species pairs using the Coleman (1999) phylogeny for which there were egg size data. Of these, ten contrasts supported the hypothesis and three did not. Binomial analysis showed that this represents a significant deviation from a random distribution of supporting and non-supporting contrasts ($p = 0.035$).

This result was reinforced by the Weighor phylogeny. We identified 11 contrasts using the same criteria for node selection as outlined above. Of these, nine supported the hypothesis. This is also a significant deviation from a random distribution ($p = 0.027$).

In summary, our results were highly sensitive to the mode of analysis and the particular phylogeny, although the general trend of the data was in favour of the PBS model. We noted that an incorrect phylogeny would be more likely to destroy rather than create the strongly significant trends that we observed in our data.

4. DISCUSSION

(a) Implications for the PBS model

We report, to the authors' knowledge, on the first phylogenetically controlled tests of the Parker *et al.* (1972) (PBS) model for the evolution of anisogamy. These tests were conducted using a published phylogeny of the Volvocales (Coleman 1999) and a phylogeny constructed using the same sequences but by a different method. Two trees were used in order to give some indication of the robustness of our results to phylogenetic inaccuracy. The results are somewhat equivocal (table 1) depending on the mode of analysis, the statistical tests used and the particular phylogeny, but they suggest acceptance of the PBS model.

Both the raw data and independent contrasts using the Weighor phylogeny supported both PBS predictions unambiguously (table 1). However, the results from independent contrasts using the Coleman (1999) phylogeny depended on the mode of analysis and statistical tests used. We noted the strikingly different results obtained with the two different trees but suggest that it would be very unlikely for the effects we observed in the Weighor analysis to have been created by a bad phylogeny. If anything, an incorrect tree would have removed any trend that existed in the data.

(b) Alternative models

While the data were broadly supportive of the PBS model, we believe that it would be unfair to suggest that this model is uniquely capable of explaining the data. Furthermore, it is not clear whether the PBS model would predict the observed pattern in the Volvocales.

Despite their role as the traditional testing ground for the PBS model (Knowlton 1974; Madsen & Waller 1983; Bell 1985), the Volvocales violate a number of its assumptions. First, the PBS model assumes 'broadcast fertilization' (*sensu* Yund 2000), i.e. gametes are released in one event, but individually into the surrounding medium. This is not what happens in the vast majority of Volvocalean species with dimorphic gametes. All but one of the species in our data set with different sized gametes release sperm in packets (see the references in electronic

Appendix B). The typical sequence of events is as follows (Iyengar & Desikachary 1981). First, colonies of both sexes clump together. This is then followed by the release of sperm packets (as opposed to individual sperm) by males, which contain up to 512 sperm.

Evidence from echinoids suggests a possible adaptive reason for releasing sperm in packets. Sperm tend to be much more short lived than eggs, presumably because of their small size (Pennington 1985; Levitan *et al.* 1991). This effect is reduced to some extent in the sea urchin *Strongylocentrotus franciscanus* if sperm are at high concentration (Levitan *et al.* 1991). The effect is termed the 'respiratory dilution effect' (Chia & Bickell 1983). Dilute sperm consume more oxygen, but the amount of oxygen consumed over the sperm's lifetime is fixed; hence, dilute sperm live longer. Swimming as a group may effectively act to increase the local sperm concentration and, hence, longevity. Adopting a collective size comparable to an egg might also make the sperm less vulnerable to predators.

Each sperm packet (effectively a dwarf male) (Bell 1985, p. 252) swims as a unit until it reaches a female colony. Here, the sperm packet enters the colony, breaking up in the process. The individual sperm then typically fertilize all the eggs therein. Rather than broadcast fertilization, it is perhaps better to regard the Volvocales as 'brooding organisms' (*sensu* Yund 2000). These are organisms in which females retain their eggs so that fertilization occurs internally with sperm that have been released into the medium.

Reproduction by a sperm packet alone may explain the trends in gamete dimorphism and egg size we observe in the Volvocales. If, as females get bigger, they place increased reproductive effort into making more eggs, then gamete dimorphism could result. Since all the sperm in a packet fertilize all the eggs in a female spheroid, males would be expected to increase the number of divisions in the packet in order to ensure that all eggs are fertilized. The data did indeed support an increase in egg number with adult size. There was a significant correlation between contrasts in $\log_{10}$ (protoplasmic volume) and egg number using both phylogenies (Weighor phylogeny, Spearman's $\rho = 0.49$, $n = 31$ and $p < 0.005$ and Coleman phylogeny, Spearman's $\rho = 0.43$, $n = 27$ and $p < 0.02$) (table 1).

The number of sperm in a packet is always 2^N , where N is the number of divisions, whereas this is not the case for egg number (see the references in electronic Appendix B). Hence, as the number of eggs and sperm increases, the inaccuracy in matching the number of eggs and number of sperm increases also, with sperm number tending to overshoot egg number by larger and larger amounts. This effect, combined with the fact that sperm packet size must probably remain within a fairly narrow size range for efficient swimming, will result in increased anisogamy. More sperm making up a similarly sized packet necessarily means smaller sperm.

Clearly, this is unlikely to represent a universal explanation for the evolution of anisogamy that would be applicable to a wide range of groups. We propose it merely as an alternative explanation that could explain the results we have presented. Indeed, as this female-driven hypothesis would predict, contrasts in the number of sperm per packet correlate very strongly with contrasts in the number of eggs to be fertilized (Spearman's $\rho = 0.65$,

$n = 17$ and $p < 0.005$). Bell (1985) reported this finding previously, but he did not take the phylogenetic relationships between species into account.

A second PBS model assumption that is violated by the Volvocales is that of no zygote provisioning or protection post-fertilization. In some species there is the potential for maternal care of the zygote because it is not released into the medium immediately. For example, in *V. c. f. kawasakiensis* the zygotes are retained in the female colony for four days before the colony disintegrates and they are released (Nozaki 1988).

What impact do our findings have on other models of the evolution of anisogamy (for a review see Hoekstra 1987)? One competing set of models attempts to explain the evolution of anisogamy as an adaptation for preventing nuclear–cytoplasmic conflict (Grun 1976; Eberhard 1980; Cosmides & Tooby 1981). The 'conflict hypothesis', as these models have collectively been called, proposes that sperm are small so that they carry a minimal amount of cytoplasm. This ensures uniparental inheritance of cytoplasmic genes (e.g. mitochondria, plastids, bacteria and intracellular symbionts) (Hurst 1990; Hastings 1992; Hurst & Hamilton 1992; Law & Hutson 1992; Frank 1996; Randerson & Hurst 1999).

It is not clear exactly what the conflict hypothesis predicts regarding the relationship between adult size and anisogamy. One argument is that multicellularity might increase the effective recessivity of a deleterious cytoplasmic variant and, hence, lead to selection for nuclear enforcement of uniparental inheritance. However, detailed modelling has shown that this is not the case (Randerson & Hurst 1999).

Another suggestion is that organisms undergoing many rounds of asexual division between sex might be less vulnerable to mitotic 'selfish' cytoplasmic variants (Hastings 1999). This might predict that multicellular organisms should be more vulnerable to 'selfish' variants that would impose selection for uniparental inheritance, although this is not clear. Our results are therefore not particularly informative in assessing the validity of the conflict hypothesis. However, we also note the theoretical difficulties associated with anisogamy as a mechanism for achieving uniparental inheritance (Randerson & Hurst 1999) and the numerous exceptions to the 'rule' that the larger gamete should donate the organelles to the zygote (Reboud & Zeyl 1994).

The authors wish to thank David Kirk and Vasiliki Koufopanou for helpful advice at an early stage in this project. They also thank Eamonn Mallon, Kate Lessells and an anonymous reviewer for constructive comments on the manuscript, Hiayoshi Nozaki for providing copies of numerous manuscripts on Volvocalean morphology and Annette Coleman for access to data on her phylogeny. L.D.H. is funded by a Royal Society Fellowship and J.P.R. is funded by a Natural Environment Research Council studentship.

REFERENCES

- Bell, G. 1978 The evolution of anisogamy. *J. Theor. Biol.* **73**, 247–270.
- Bell, G. 1985 The origin and early evolution of germ cells as illustrated by the Volvocales. In *Origin and evolution of sex* (ed. H. O. Halvorson & A. Monroy), pp. 221–256. New York: Allan R. Liss.

- Bruno, W. J., Socci, N. D. & Halpern, A. L. 2000 Weighted neighbor joining: a likelihood-based approach to distance-based phylogeny reconstruction. *Mol. Biol. Evol.* **17**, 189–197.
- Bulmer, M. 1994 *Theoretical evolutionary ecology*. Sunderland, MA: Sinauer Associates.
- Chia, F.-S. & Bickell, L. R. 1983 Echinodermata. In *Reproductive biology of invertebrates, volume II: spermatogenesis and sperm function* (ed. K. G. Adiyodi & R. G. Adiyodi), pp. 545–620. New York: John Wiley & Sons.
- Coleman, A. W. 1999 Phylogenetic analysis of 'Volvocae' for comparative genetic studies. *Proc. Natl Acad. Sci. USA* **96**, 13 892–13 897.
- Cosmides, L. M. & Tooby, J. 1981 Cytoplasmic inheritance and intragenomic conflict. *J. Theor. Biol.* **89**, 83–129.
- Cox, P. A. & Sethian, J. A. 1985 Gamete motion, search, and the evolution of anisogamy, oogamy and chemotaxis. *Am. Nat.* **125**, 74–101.
- Dusenbery, A. B. 2000 Selection for high gamete encounter rates explains the success of male and female mating types. *J. Theor. Biol.* **202**, 1–10.
- Eberhard, W. G. 1980 Evolutionary consequences of intracellular organelle competition. *Q. Rev. Biol.* **55**, 231–249.
- Frank, S. A. 1996 Host–symbiont conflict over the mixing of symbiotic lineages. *Proc. R. Soc. Lond. B* **263**, 339–344.
- Grun, P. 1976 *Cytoplasmic genetics and evolution*. New York: Columbia University Press.
- Hastings, I. M. 1992 Population genetic-aspects of deleterious cytoplasmic genomes and their effect on the evolution of sexual reproduction. *Genet. Res.* **59**, 215–225.
- Hastings, I. M. 1999 The costs of sex due to deleterious intracellular parasites. *J. Evol. Biol.* **12**, 177–183.
- Hoekstra, R. F. 1982 On the asymmetry of sex: evolution of mating types in isogamous populations. *J. Theor. Biol.* **98**, 427–451.
- Hoekstra, R. F. 1987 The evolution of sexes. In *The evolution of sex and its consequences* (ed. S. C. Stearns), pp. 59–91. Basel, Switzerland: Birkhäuser.
- Hurst, L. D. 1990 Parasite diversity and the evolution of diploidy, multicellularity and anisogamy. *J. Theor. Biol.* **144**, 429–443.
- Hurst, L. D. & Hamilton, W. D. 1992 Cytoplasmic fusion and the nature of sexes. *Proc. R. Soc. Lond. B* **247**, 189–194.
- Iyengar, M. O. P. & Desikachary, T. V. 1981 *Volvocales*. New Delhi: Council for Agricultural Research.
- Knowlton, N. 1974 A note on the evolution of gamete dimorphism. *J. Theor. Biol.* **46**, 283–285.
- Law, R. & Hutson, V. 1992 Intracellular symbionts and the evolution of uniparental cytoplasmic inheritance. *Proc. R. Soc. Lond. B* **248**, 69–77.
- Levitan, D. R., Sewell, M. A. & Fu-Shiang, C. 1991 Kinetics of fertilization in the sea urchin *Strongylocentrotus franciscanus*: interaction of gamete dilution, age, and contact time. *Biol. Bull.* **181**, 371–378.
- Madsen, J. D. & Waller, D. M. 1983 A note on the evolution of gamete dimorphism in algae. *Am. Nat.* **121**, 443–447.
- Maynard Smith, J. 1978 *The evolution of sex*. Cambridge University Press.
- Nozaki, H. 1988 Morphology, sexual reproduction and taxonomy of *Volvox carteri* f. *kawasakiensis* f. nov. (Chlorophyta) from Japan. *Phycologia* **27**, 209–220.
- Parker, G. A., Baker, R. R. & Smith, V. G. F. 1972 The origin and evolution of gamete dimorphism and the male–female phenomenon. *J. Theor. Biol.* **36**, 181–198.
- Pennington, J. T. 1985 The ecology of fertilization of echinoid eggs: the consequence of sperm dilution, adult aggregation, and synchronous spawning. *Biol. Bull.* **181**, 261–268.
- Purvis, A. & Rambaut, A. 1995 Comparative analysis by independent contrasts (CAIC): an Apple Macintosh application for analysing comparative data. *Comp. Appl. Biosci.* **11**, 247–251.
- Randerson, J. P. & Hurst, L. D. 1999 Small sperm, uniparental inheritance and selfish cytoplasmic elements: a comparison of two models. *J. Evol. Biol.* **12**, 1110–1124.
- Reboud, X. & Zeyl, C. 1994 Organelle inheritance in plants. *Heredity* **72**, 132–140.
- Strimmer, K. & Von Haeseler, A. 1996 Quartet puzzling: a quartet maximum-likelihood method for reconstructing tree topologies. *Mol. Biol. Evol.* **13**, 964–969.
- Yund, P. O. 2000 How severe is sperm limitation in natural populations of marine free-spawners? *Trends Ecol. Evol.* **15**, 10–13.

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