THE ROYAL SOCIETY

Sexual selection and speciation in mammals, butterflies and spiders

Matthew J. G. Gage[1*], Geoffrey A. Parker[2], Soren Nylin[3] and Christer Wiklund[3]

[1]*Centre for Ecology, Evolution and Conservation, School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK*
[2]*Population and Evolutionary Biology Research Group, School of Biological Sciences, University of Liverpool, Liverpool L69 3GX, UK*
[3]*Department of Zoology, University of Stockholm, Stockholm, Sweden*

Recently refined evolutionary theories propose that sexual selection and reproductive conflict could be drivers of speciation. Male and female reproductive optima invariably differ because the potential reproductive rate of males almost always exceeds that of females: females are selected to maximize mate 'quality', while males can increase fitness through mate 'quantity'. A dynamic, sexually selected conflict therefore exists in which 'competitive' males are selected to override the preference tactics evolved by 'choosy' females. The wide variation across taxa in mating systems therefore generates variance in the outcome of intrasexual conflict and the strength of sexual selection: monandry constrains reproductive heterozygosity and allows female choice to select and maintain particular (preferred) genes; polyandry promotes reproductive heterozygosity and will more likely override female choice. Two different theories predict how sexual selection might influence speciation. Traditional ideas indicate that increased sexual selection (and hence conflict) generates a greater diversity of male reproductive strategies to be counteracted by female mate preferences, thus providing elevated potentials for speciation as more evolutionary avenues of male–female interaction are created. A less intuitively obvious theory proposes that increased sexual selection and conflict constrains speciation by reducing the opportunities for female mate choice under polyandry. We use a comparative approach to test these theories by investigating whether two general measures of sexual selection and the potential for sexual conflict have influenced speciation. Sexual size dimorphism (across 480 mammalian genera, 105 butterfly genera and 148 spider genera) and degree of polyandry (measured as relative testes size in mammals (72 genera) and mating frequency in female butterflies (54 genera)) showed no associations with the variance in speciosity. Our results therefore show that speciation occurs independently of sexual selection.

Keywords: size dimorphism; sperm competition; mating pattern; sympatric

1. INTRODUCTION

Darwin (1871) proposed that sexual selection and divergence in female choice was an important stage through which species could become reproductively isolated. Experimental evidence shows that divergence and subsequent assortative mating-preference genotypes can evolve (e.g. Houde & Endler 1990; Andersson 1994; McMillan *et al.* 1997). In this study, we explore whether sexual selection, that leads to male–female conflict over reproductive optima, could influence the potential for reproductive isolation and new species to evolve, by measuring generic species diversity in relation to variance in measures of the strength of sexual selection and conflict across mammals, butterflies and spiders.

Sexual selection is recognized as a variable force that can facilitate reproductive isolation and speciation (e.g. Turner & Burrows 1995; McMillan *et al.* 1997; Seehausen & von Alphen 1999). In taxa where males have been under more intense sexual selection leading to greater variance in reproductive success across males, spe-

ciation can be encouraged via Fisherian runaway divergence, leading to more rapid isolation of populations (Lande 1981). Recently, this phenomenon has been explored in more detail by considering that sexual conflict between males and females, as a consequence of sexual selection in general, can exert an effect. Parker (1979) identified the potential for reproductive conflict between males and females to influence speciation over 20 years ago, and there has been a recent resurgence in theoretical attention to the possibility that speciation could be influenced by sexual selection and conflict via a number of routes (Parker & Partridge 1998; Arnqvist *et al.* 2000; Gavrilets 2000; Gavrilets *et al.* 2001).

Sexual conflict is a fundamental phenomenon within the general force of sexual selection. Conflict between male and female reproductive interests has been recognized at a number of different levels from whole organism behavioural divergence to intra-locus genomic conflicts. At the mating strategy level, Bateman's (1948) classic *Drosophila* experiment exemplifies the fundamental differences in selection acting on male and female reproductive optima: males are limited by numbers of matings while females are limited by offspring production. More recent work on *Drosophila melanogaster* shows that males produce

*Author for correspondence (m.gage@uea.ac.uk).

Proc. R. Soc. Lond. B (2002) **269**, 2309–2316
DOI 10.1098/rspb.2002.2154

2309

© 2002 The Royal Society

seminal substances that are toxic to females (Chapman *et al.* 1995), and that relaxation of sexual selection through enforced monogamy leads to less 'harmful' males (Holland & Rice 1999).

Males almost always invest less in direct progeny production than females (Trivers 1972). Males therefore invariably inherit a reproductive strategy with an intrinsically greater potential reproductive rate than females (Clutton-Brock & Parker 1992; see Gwynne & Simmons (1990) for an exception of role-reversal), leading to a male-biased operational sex ratio (Emlen & Oring 1977). Male reproductive success will therefore usually be strongly influenced by the quantity of mates, while female success depends more upon mate quality. This asymmetry leads to sexual selection generally acting on males, and leads to sexual conflict over mating optima: males can be selected to mate with females that are selected to reject them, and males typically have a higher optimal mating frequency than females (Parker 1979). Clutton-Brock & Parker (1995) theoretically reviewed the abundant evidence that females suffer forced copulation, harassment, intimidation and punishment in a wide variety of taxa and that this coercion is costly for females. There is some evidence that females can gain benefits from multiple mating (e.g. Andersson 1994; Tregenza & Wedell 2002), however, because males possess a greater potential reproductive rate and females can suffer costs from mating, polyandry is almost always more beneficial for male fitness than for females (Parker 1979; Clutton-Brock & Parker 1995).

Recent theoretical studies have addressed the potential for sexual selection and conflict to be a catalyst, rather than a product, of evolution by playing a driving role in speciation. It is clear that mate choice and reproductive isolation are important components in the speciation process and, although there are examples of species recognition driven by male choice (Jiggins *et al.* 2001), it is invariably the female that evolves a mating preference for male traits (Andersson 1994). Females have a lower reproductive potential, and therefore will be more discriminatory than males. When sexual selection is elevated, there is greater potential for different avenues of speciation to evolve and for barriers between hybrids to evolve (van Doorn *et al.* 1998). Because females tend to be the 'choosy' sex, when hybridization generates marginal costs, selection will act more intensively on females to be the selective sex. By contrast, under situations of incipient speciation, males (with a greater potential reproductive rate) will be less discriminatory. This male–female phenomenon exemplifies the fundamental conflict between male and female reproductive interests within sexual selection. Recent theoretical attention (Gavrilets 2000; Gavrilets *et al.* 2001) has been paid to sexual conflict and speciation by considering asymmetries in any male–female conflict from a quantitative genetics approach, such as an intralocus conflict over inheritance and expression of genes which benefit one sex but not the other (Rice & Holland 1997). Gavrilets (2000) and Gavrilets *et al.* (2001) theorize that an enhanced male–female arms race is generated when sexual conflict intensifies (see also Parker 1979, 1983). The resultingly increased adaptations and counter-adaptations facilitate an increased diversity of male–female reproductive strategies from

which reproductive isolation, and therefore speciation, more readily evolves. Evidence for such increased dynamism comes from the diversity of male genitalia evolution in monandrous and polyandrous clades (Arnqvist 1998).

Two comparative studies test the theory that enhanced sexual selection/conflict leads to higher speciation levels. In passerine birds, species diversity is higher in sister groups where sexual selection has increased sexual dichromatism (Barraclough *et al.* 1995). Arnqvist *et al.* (2000) examined more direct sexual conflict effects on speciation by examining monandry–polyandry. In a meta-analysis of mating patterns and speciation across insects Arnqvist *et al.* (2000) showed that polyandrous taxa were four times more speciose than their most closely related monandrous sister taxa. This study therefore provides support for the hypothesis that increased sexual selection and conflict through polyandry encourages speciation.

In contrast to the traditional predictions that sexual selection (Turner & Burrows 1995; van Doorn *et al.* 1998; Higashi *et al.* 1999) and sexual conflict (Gavrilets 2000; Gavrilets *et al.* 2001) generate higher levels of speciation, a more counterintuitive theory (Parker 1983) predicts a contrasting effect. Parker & Partridge (1998) used an evolutionarily-stable-strategy-based approach to consider how asymmetries in reproductive interests could lead to variance in the promotion or prevention of reproductive isolation. In taxa where males are successful in overcoming optimal female reproductive interests and preferences (i.e. males are 'winning' mating conflicts and inducing matings against female resistance), then speciation will be hindered because specific male-trait–female-preference associations that could eventually lead to reproductive isolation are constrained from evolving. By contrast, when females mate at their own optima, specific preferences cannot be overridden by the enhanced male urgency to mate. Female resistance and mate choice are therefore able to persist and evolve (rather than being diluted/mixed at each generation), eventually leading to reproductive isolation (Parker & Partridge 1998). In other words, the Parker and Partridge model hypothesizes that conflict either constrains or promotes speciation depending upon where the mating pattern resolves relative to the male or female reproductive optima; patterns close to theoretical female optima promote speciation through the evolution and maintenance of trait–preference associations, while patterns close to male optima constrain speciation by the constant overriding of female preferences and therefore genotypic dilution and intermixing of potential reproductive isolation avenues. The guppy (*Poecilia reticulata*) system exemplifies how this model might apply: populations are clearly differentiated and locally adapted (Magurran 1998) but no reproductive isolation has occurred despite controlled experiments revealing the potential for female mate choice (Houde & Endler 1990). In this fish, however, males continually harass females for matings and succeed in forced copulation, and this successful male strategy may circumvent female trait-preference avenues and constrain reproductive isolation (Magurran 1998). Parker & Partridge (1998) therefore dissect apart general variance in sexual selection to consider how male–female conflict within sexual selection could affect speciation.

In this study, we test the sexual selection and conflict

models by exploring comparative variance in speciation across mammals, butterflies and spiders. We use two different measures of sexual selection that could lead to reproductive conflict given the fundamental differences between male and female interests. The important measure we need to establish is where the evolved mating pattern lies relative to the male and female reproductive optima; such optima are probably dynamic and difficult to identify; however, an almost consistent condition is that male reproductive potential exceeds that of females. Sexual selection therefore drives males to evolve strategies that differ from female optima and this divergence presents variance in sexual conflict. We adopt an approach exploring general and consistent measures of sexual selection and potential conflict. We acknowledge that there will be exceptions to our general predictions, but our broad analyses explore species diversity in relation to measures of sexual selection that quantify mating patterns and reproductive heterozygosity, and the potential for males to override female mate choice criteria.

Conflict can generate two types of interrelated costs to females: either mating with an inferior/non-preferred male (indirect or genetic costs), or mating with excessive males (direct costs). Mating with non-preferred or too many males is a particular risk when males are bigger than females. Sexual size dimorphism can evolve for various reasons (see Andersson (1994) and § 4), but a number of studies have shown that relatively enlarged male body size can lead to female mating preferences being overridden by simple physical asymmetries (Smuts & Smuts 1993; Clutton-Brock & Parker 1995). Accordingly, the degree of male-biased sexual size dimorphism is likely to be an informative predictor of the potential for generating a mating pattern closer to male optima. It is well documented that sexual selection can drive sexual size dimorphism (e.g. Lande 1980; Lindenfors & Tullberg 1998) and there is good evidence that body size asymmetries allow sexual coercion and conflict to operate during reproduction (Smuts & Smuts 1993; Clutton-Brock & Parker 1995). We therefore use sexual size dimorphism as one fundamental and universal measure of the potential for male–female conflict over mating optima, which enables representative taxonomic breadth to be analysed across mammals, butterflies and spiders. In many invertebrates females are bigger than males due to selection on fecundity; however, the relative size of the male to the female across taxa remains a useful predictor of the potential for males to combat female mate choice preferences. Spiders provide a particularly good example of a taxon where female choice cannot be overridden by non-preferred males as cannibalism is an established risk (Samu *et al.* 1999), while mating patterns in many taxa involve male physical coercion of females where larger male body size may be an advantage (Elgar 1998).

We further investigate levels of speciation in relation to variance in polyandry across mammals and butterflies using two measures that are strongly correlated with the evolved mating pattern: relative testis size in mammals and mean spermatophore count in butterflies. (Simple observational measures of mating pattern are not necessarily objective and natural reproductive behaviour, particularly successful mating, is notoriously difficult to study in detail). Level of polyandry dictates the probability of mul-

tiple-male gene flow through the population. When multi-male gene flow persists, there may be increased potential for speciation due to greater genotypic variance (Gavrilets 2000; Gavrilets *et al.* 2001); alternatively, females may be less able to evolve reproductive isolation mechanisms (Parker 1979; Parker & Partridge 1998). An almost universal correlate of polyandry that is supported by a range of taxa (see Parker *et al.* (1997) which reviews 12 of 14 comparative studies concurring) is the relative (to male body weight) size of the testes: polyandrous taxa are selected to increase investment in testis size to produce more sperm for increased sperm competition and mating opportunities. Testis size has been described as the 'witness of the mating system' (Short 1997). Accordingly, we use relative testis size as an index of polyandry for comparison across mammalian genera in relation to speciation.

Butterflies also provide an excellent system for exploring mating patterns because the male produces a spermatophore inside the female that persists (in most species) for the lifetime (and beyond) of the insect (Eberhard 1985; Svärd & Wiklund 1989). It is therefore possible to sample wild populations and quantify the degree of polyandry sustained by that species. Spermatophore counts have been described as 'the best field data on remating frequencies' (Eberhard 1985) and provide an objective quantification of natural mating pattern which we can explore in relation to speciation across genera. In some butterfly taxa, there is good evidence that females can benefit nutritionally from spermatophore receipt (e.g. *Pieris napi*, Wiklund *et al.* 1998) and therefore some degree of polyandry. However, even in *P. napi* where polyandry clearly benefits females directly (Wiklund *et al.* 1998), the male mating potential is significantly greater than the female natural mating frequency: *P. napi* males have a calculated potential reproductive rate that is 8–13 times greater than females (Wiklund *et al.* 1998), despite evidence that female egg production benefits from some degree of multiple mating. Indeed, across seven temperate butterfly species with *ad libitum* mating opportunities, the male mating frequency always exceeded that of females (C. Wiklund, unpublished data). Apart from rare examples of role-reversed taxa (Gwynne & Simmons 1990), the optimal mating frequency for males is greater than the level of polyandry, even in taxa where some degree of polyandry clearly benefits females. In monandrous taxa, we can conclude that females are not mating at the male optimum while in polyandrous taxa there is greater potential for mating patterns to have approached male optima. Sexual selection therefore evolves a realized mating pattern which quantifies the risk of multi-male gene flow through populations, and signals the potential position of the mating pattern relative to male and female optima.

2. METHODS

(a) *Data collation*

Data were collated from the literature (see electronic Appendix A available on The Royal Society's Publications Web site). Mammalian body weights were derived from the CRC handbook of mammalian body masses (Silva & Downing 1995). To avoid inter-population variance, only studies of species that cited both male and female weights from the same population were used to produce measures of sexual size dimorphism. Combin-

ing all reliable data on male and female body weights, we were able to produce mean male and mean female body weights for 480 genera. Numbers of species per genus were counted from 'mammals of the world' (Corbet & Hill 1991). Sexual size dimorphism for each genus was calculated as the residual mean male weight derived from a total linear regression of male on female body weights across 480 genera. Testis weight was derived from various literature sources (Kenagy & Trombulak 1986; Ginsberg & Rubenstein 1990; Harcourt 1991; Hosken 1997, 1998) and species values were collated into generic means. Potentially confounding allometric relationships between organ and body weight were controlled for by calculating residual testis weight from the body weight regression across mammalian genera. (Body weights in this regression were only those male weights provided in the same testis-size literature sources.)

We concentrated on the butterfly fauna of Papua New Guinea as this has recently been described in consistent detail by Parsons (1999). Furthermore, the island of Papua New Guinea represents a metapopulation model with sympatric potential for exploring diversity associations within this diverse fauna. Body size was measured as forewing span (wing span scales significantly with body weight in Lepidoptera (e.g. Morrow & Gage 2000)). Again, generic means for male and female body size were calculated and sexual size dimorphism was the residual male wing span derived from the regression of male and female generic wing spans. The number of species per genus in the Papua New Guinean butterfly fauna were derived from Parsons (1999). Spermatophore count data were compiled from the literature for 54 genera (data in Svärd & Wiklund (1989), reviewed in Drummond (1984)) (sample size per genus averaged 99 individuals and ranged from 10 to 1111 mated females). Mean spermatophore count was calculated for each represented butterfly genus. Because authorities differ in their interpretation of global butterfly taxonomic organization, the total number of global species per genus was determined from two compilations and analysed twice. First, we compiled a world list largely using Smart (1975), with more recent modifications for some genera as reported by specific authorities, applied conservatively with respect to the number of 'good' species (Scott 1986; DeVries 1987; Larsen 1991; Corbet & Pendlebury 1992; Spencer-Smith *et al.* 1994; Tuzov 1997, 2000; Wahlberg 2000; Nylin *et al.* 2001). Second, we derived global species number for our 54 genera from www.funet.fi/pub/sci/bio/life/intro.html, which has also collated data from published texts, reports, museum collections and personal communications with relevant experts. The main difference between the datasets is that in the more conservative former organization, fewer species are recognized in most genera. In some instances the datasets also differ in how genera are delimited.

We analysed the spider fauna of Britain and Northern Europe which has been consistently described in detail (Roberts 1996). Again, generic means for male and female body size were calculated and sexual size dimorphism was the residual male body length derived from the regression of male and female generic body lengths.

(b) *Comparative analyses*

Species-level analyses can be misleading because of phylogenetic non-independence and evolutionary inertia (Harvey & Pagel 1991). In this study, we adopt different approaches to control for phylogenetic association depending upon the taxon under investigation and its established phylogenetic information. Prim-

arily, we explore all relationships between traits at the genus level (because speciosity is measured as the number of species that have evolved within a genus) and this exercises a level of control for phylogenetic non-independence as generic relationships will be less constrained by association than comparisons between species (Harvey & Pagel 1991). However, for all analyses we exercise further control on potential confounds from phylogeny. The mammalian phylogeny is relatively well described and we are therefore able to use comparative analysis by independent contrasts (CAIC, v. 2) (Purvis & Rambaut 1994). CAIC generates contrasts in trait values at independent events from a user-coded phylogeny. For species diversity analyses in mammals we also use MACROCAIC (a modified version of CAIC (Agapow & Isaac 2002)), which is specifically designed for analysing patterns of variance in clade richness. MACROCAIC is increasingly conservative because multiple nodes in the phylogeny (where clade size could be variable) generate no contrast values, thus substantially decreasing the degrees of freedom. The mammalian phylogeny was coded by combining Novacek's (1992) higher level relationships with Corbet & Hill's (1991) lower level classifications. Because there is variance in the details of phylogenetic information between mammalian families, we maintained consistency and used a speciational model of evolutionary change, where all branch length segments are equal (Harvey & Pagel 1991). To check for assumptions concerning the rate of evolutionary change in character values and that appropriate transformations had been applied (Purvis & Rambaut 1994), we regressed absolute contrast values on their nodal estimates: all regression slopes were not significantly different from zero, apart from one regression of male and female body weight across 480 genera (107 contrasts) where $p = 0.037$. However, as multiple comparisons were performed within the analysis of absolute contrasts and nodal values, Bonferroni correction would mark this marginal result as non-significant by chance due to type I error.

Butterflies and spiders do not have the well-documented chronological phylogeny of the mammals. We therefore remove potential effects of phylogenetic inertia among lower-level taxa by using phylogenetic subtraction (Stearns 1983) within family-level clades (whose existence and organization are more accepted (e.g. Ackery 1984; Roberts 1996)). Essentially, any inertia associated with phylogenetic relatedness below the family level is removed by partialing away the mean character value for a family from each of its represented genera. The sum value of variance resulting from phylogenetic or taxonomic affiliation within each family is therefore reduced to zero and generic values are independent of effects of phylogenetic association following evolutionary radiation beyond the family level. These analyses are unable to explore associations deeper in the phylogenetic tree because calculated trait values at older points in the tree would be unreliable; therefore only more recently evolved trait values are analysed.

All trait values were log-transformed prior to analysis (Harvey & Pagel 1991). To control for potentially confounding continuous associations (such as allometric correlations between species number and generic body sizes) we calculate residual values from any significant primary regressions before secondary analysis.

3. RESULTS

Our analysis results showed consistency irrespective of the method of phylogenetic control: we found no associ-

ations between sexual size dimorphism and numbers of species per genus across 480 mammal, 105 butterfly, and 148 spider genera. We also found no evidence for associations between polyandry (as measured by residual testes size or spermatophore count) and speciosity in 72 mammalian and 54 butterfly genera. We therefore find no evidence for any effect of sexual conflict over mating pattern on speciation in spiders, butterflies or mammals.

(a) Mammals

Mammalian genera with heavier body weights contained fewer species ($r^2 = 0.038$, $p = 0.046$, $n = 107$ CAIC contrasts) and this relationship was also significant at the genus level of analysis ($r^2 = 0.07$, $p < 0.0001$, $n = 480$). However, analysis with MACROCAIC did not show any relationship between body mass and clade size ($r^2 = 0.004$, $p = 0.69$, $n = 40$ MACROCAIC contrasts). Accordingly, we avoid any potential autocorrelations in body weight analyses by controlling for this body mass relationship and standardize to residual species number per genus before subsequent generic and CAIC analyses, but not using MACROCAIC analysis. (It is important to note that our speciosity results for the mammalian analyses were consistently nonsignificant irrespective of whether the absolute body size or clade size association was controlled for.)

Male and female body weights were strongly correlated across 480 mammalian genera ($r^2 = 0.987$, $p < 0.0001$, $n = 480$ genera, relationship: $y = 1.024x - 0.003$; $r^2 = 0.9$, $p < 0.0001$, $n = 107$ CAIC contrasts, relationship: $y = 0.99x - 0.069$; $r^2 = 0.99$, $p < 0.001$, $n = 40$ MACROCAIC contrasts, relationship: $y = 1.04x + 0.01$). We calculate residual male body weights from these relationships to produce a measure of sexual dimorphism for each genus.

Sexual size dimorphism (residual male body weight) was not associated with the residual number of species per genus across 480 mammalian genera ($r^2 = 0.004$, $p = 0.18$, $n = 480$ genera; $r^2 = 0.098$, $p = 0.31$, $n = 107$ contrasts; $r^2 = 0.002$, $p = 0.76$, $n = 40$ MACROCAIC contrasts).

Male body size was allometrically associated with testes weight across 72 mammalian genera ($r^2 = 0.9$, $p < 0.0001$, $n = 72$ genera; $r^2 = 0.44$, $p = 0.0003$, $n = 26$ contrasts) and the slopes showed characteristic negative allometry ($y = 0.76x + 2.88$ (genus relationship) and $y = 0.57x + 0.027$ (contrasts relationship)) for organ–body weight relationships. We therefore control for this allometry and calculate residual testes weight as a relative measure of the mating pattern sustained by a genus. There was no association between residual testes weight and residual numbers of species per genus across 72 genera ($r^2 = 0.025$, $p = 0.18$, $n = 72$ genera; $r^2 = 0.08$, $p = 0.16$, $n = 26$ contrasts; $r^2 = 0.14$, $p = 0.47$, $n = 6$ MACROCAIC contrasts) revealing no tendency for mating pattern to influence speciation across mammals.

We regressed residual testes weight ('mating pattern') on residual male weight ('sexual size dimorphism') across 72 genera but found no evidence for any relationship between size dimorphism and mating pattern ($r^2 = 0.011$, $p = 0.37$, $n = 72$ genera; $r^2 = 0.0001$, $p = 0.96$, $n = 26$ CAIC contrasts).

(b) Butterflies

We found no evidence across 105 Papua New Guinean butterfly genera that taxa with larger body sizes (as meas-

ured by forewing span) contained fewer species ($r^2 = 0.0018$, $p = 0.66$, $n = 105$ genera) and so used absolute species number per genus in subsequent analyses.

Male and female forewing spans were strongly correlated across 105 genera ($r^2 = 0.98$, $p < 0.0001$, $n = 105$, relationship: $y = 0.924x + 0.24$) and we calculate sexual size dimorphism as the residual male values per genus from this regression.

Sexual size dimorphism (residual male wing span) was unrelated to the numbers of species per genus across 105 butterfly genera ($r^2 = 0.01$, $p = 0.44$, $n = 105$ genera; $r^2 = 0.004$, $p = 0.49$, $n = 105$ phylogenetically subtracted generic values). Mean spermatophore count was also not associated with numbers of species per genus across 54 worldwide butterfly genera using two speciosity estimates as described in § 2 ($r^2 = 0.0005$, $p = 0.87$, $n = 54$ genera and $r^2 = 0.011$, $p = 0.45$, $n = 54$ genera) and phylogenetic subtraction did not affect this finding ($r^2 = 0.017$, $p = 0.36$, $n = 53$ and $r^2 = 0.027$, $p = 0.24$, $n = 53$).

(We were not able to regress size dimorphism on spermatophore count across the global butterfly spermatophore count dataset because there are insufficient precise measures of male/female body size for those species with spermatophore count data.)

(c) Spiders

We found no evidence across 148 spider genera of northern Europe that taxa with larger body sizes (as measured by body length) contained fewer species ($r^2 = 0.003$, $p = 0.53$, $n = 148$) and so used absolute species number per genus in subsequent analyses.

Male and female body lengths were correlated across 148 genera ($r^2 = 0.86$, $p < 0.0001$, $n = 148$, relationship: $y = 0.833x + 0.058$) and we calculate sexual size dimorphism as the residual male values per genus from this regression.

Sexual size dimorphism (residual male body length) showed no association with the numbers of species per genus either at the generic level ($r^2 = 0.006$, $p = 0.36$, $n = 148$) or after phylogenetic subtraction ($r^2 = 0.006$, $p = 0.38$, $n = 130$).

4. DISCUSSION

Our comparative analyses lend no support for the theory that sexual selection, multi-male gene flow and potential conflict over mating pattern and mate choice have influenced speciation. Speciosity is unaffected by the relative sizes of males to their conspecific females in three major taxa. Mating pattern and level of polyandry are also not associated with the numbers of species that have evolved within a genus across mammals and butterflies.

There are a number of interpretations for our results but, importantly, it seems highly improbable that non-significance arose due to lack of statistical power. Our comparative analyses were based on relatively large datasets (data on species within 480 mammalian, 105 butterfly and 148 spider genera for size dimorphism analyses; and 72 mammalian and 54 nymphalid butterfly genera for mating pattern analyses; see electronic Appendix A) using three taxonomic groups and two measures of the potential for sexual selection leading to the potential for multi-male gene flow, and conflict within mating patterns and mate

choice. It is possible that sexual size dimorphism or measures of polyandry do not actually relate to sexual conflict over evolved mating patterns. However, polyandry is a direct measurement of the potential for multi-male gene flow within populations, and there is much evidence from both within-species and comparative studies that these measures are likely indicators of the existence of, or at least potential for, mating conflict between females and males (Smuts & Smuts 1993; Clutton-Brock & Parker 1995). Male-biased sexual size dimorphism is also associated with mating systems across haplorhine primates (Lindenfors & Tullberg 1998) and butterflies (Wiklund & Forsberg 1991). Sexual size dimorphism can clearly be selected for by male–male competition and/or female mate choice (Andersson 1994). However, given that male potential reproductive rate and mating frequency invariably exceed that of females, we would expect there to be parallel selection in such cases on males to use any size advantage to mate closer to their own optimal frequency (which will invariably be greater than that of the females).

Measures of mating pattern are likely to indicate the level of sperm competition and therefore the representation of different males' sperm, or genes, at fertilization (Birkhead & Møller 1998) and the probability that males have driven the mating pattern above the female optimum. This measure therefore predicts the position that the mating pattern occupies relative to theoretical male and female optima. We cannot envisage practical measures of sexual selection and conflict over mating frequency that are more informative than sexual size dimorphism or mating pattern, and which could be analysed using a global macroecological approach with such representative datasets.

Our findings differ from two other comparative studies exploring speciation in relation to sexual conflict. In a comparative study of passerine birds, there is evidence that species diversity is higher in sister groups where sexual selection has increased sexual dichromatism (Barraclough *et al.* 1995; Owens *et al.* 1999). Although these analyses indicate a link between speciation and sexual selection in general, it is not clear how the results might fit with sexual conflict models, because the true measure of mating pattern conflict is the degree of polyandry (or the intersexual asymmetry in the existing mating frequency optima and therefore relative flow of non-preferred male genes). Sexual dichromatism is one phenotypic result of sexual selection, but it is not clear how such male traits influence polyandry. Arnqvist *et al.* (2000) focused more directly on sexual conflict arising from mating pattern itself, and found that polyandrous insect genera contained four times as many species as monandrous relatives. Arnqvist *et al.* (2000) conducted an analysis with a theoretical background that sought only to explore monandry versus polyandry contrasts as the potential for sexual conflict. Accordingly, our sexual size dimorphism analyses may have little relevance to the Arnqvist *et al.* (2000) findings. However, our results analysing mating pattern (spanning monandry to polyandry) in butterflies and mammals are relevant to the results of Arnqvist *et al.* (2000). As our analyses are restricted to two single taxa (mammals and butterflies), it is possible that our study does not have the breadth of Arnqvist *et al.* who explored

across all insects using 25 contrasts. However, while our study lacks such breadth across insects, we have explored across both taxa in depth using two accepted measures of degree of polyandry. Furthermore, our general analyses reduce any specific bias from other forces (such as ecology) known to drive speciation to confound our results. Arnqvist *et al.* (2000) used comparisons between groups that varied in their degree of relatedness (because of the constraint from variable information in mating pattern across insects) to produce 25 contrasts. There is increased potential for these specific comparisons across the more variant Insecta to be influenced by specific differences in, for instance, niche availability (and hence potential for incipient speciation) between sister groups. Another contrast with Arnqvist *et al.* (2000) is that we use all the variance in speciosity and measures of sexual conflict in regression-based analyses; this allows us to determine both whether there is an effect, and also the magnitude of the effect. Arnqvist *et al.* (2000) analyse only whether or not results go in the predicted direction, and results will also be heavily influenced by parametric contrasts of taxa exhibiting major differences. Interestingly, these authors found the strongest support from the butterflies, which is the taxon we studied in more detail here. One possibility is that the driving effects on speciation of either conflict theory (which are predicted to go in opposite directions) effectively cancel one another out. If both processes generate reproductive isolation, then speciosity in polyandrous clades may be more or less diverse than monandrous taxa, resulting in no perceptible differences.

Our findings could be explained if sexual selection and conflict over mating pattern is an effect, rather than a cause, of incipient speciation arising due to other, more profound, forces; for example, if expanding clades tend to evolve polyandry. Thus, the mating pattern will be a consequence, not a driver, of speciation. There is also the possibility for traditional taxonomic methods to be confounded by an indirect signal of mating pattern so that apparent levels of speciosity are confounded by morphological cladistics (if, for example, mating pattern and sexual selection relate to morphology, which determines how species in clades are organized). One means to explore this potential would be to compare genetic relatedness of species in comparable monandrous and polyandrous genera.

Selection and conflict at the mating level is paralleled at the gametic level by sperm competition and cryptic female choice (Parker 1970; Eberhard 1996; Stockley 1997; Birkhead & Møller 1998). There is potential for females to override reproductive actions at the whole animal level with adaptations at the cryptic level of the gamete. Accordingly, situations where males are potentially overriding female mate choice and multiply mating may not translate into actual multi-male gene flow because of cryptic female choice and control. There is good evidence, for example, in sperm competitions between *Chorthippus parallelus* subspecies, that homogamic (within-subspecies) fertilizations were more successful than heterogamic (hybrid) fertilizations, indicating female preference for homogamous fertilizations and/or decreased competitiveness of sperm from hybrid males (Hewitt *et al.* 1989). Detailed examinations of sperm–egg interactions in *D.*

melanogaster show that gametic incompatibilities between races could be an isolating mechanism during incipient speciation (Alipaz *et al.* 2001). The environment of the female reproductive tract presents a range of opportunities for females to evolve adaptations that override reproductive activities at the whole animal level (Eberhard 1985; Stockley 1997; Birkhead & Møller 1998). Nevertheless, the very fact that males continue to show persistence against female resistance implies that males succeed against cryptic processes, at least some of the time, and clear empirical demonstrations of cryptic female choice have yet to differentiate between male and female effects (e.g. Simmons *et al.* 1996).

In summary, we find no evidence that two measures of sexual selection, which potentially lead to variance in multi-male gene flow and sexual conflict, are associated with the degree of speciation across mammalian, butterfly and spider genera. If sexual size dimorphism and/or measures of mating pattern are predictors of the strength of sexual selection, and the level of multi-male gene flow or status of male–female conflict, we find that speciation occurs independently of sexual selection, and rather via naturally selected processes of speciation that are well recognized (Mayr 1963).

REFERENCES

Ackery, P. R. 1984 Systematic and faunistic studies of butterflies. In *The biology of butterflies* (ed. R. I. Vane-Wright & P. R. Ackery), pp. 9–21. London: Academic.

Agapow, P.-M. & Isaac, N. J. B. 2002 MacroCaic: correlations of species richness. *Diversity and Distributions*. (In the press.)

Alipaz, J. A., Wu, C.-I. & Karr, T. L. 2001 Gametic incompatibilities between races of *Drosophila melanogaster*. *Proc. R. Soc. Lond.* B **268**, 789–795. (DOI 10.1098/rspb.2000.1420.)

Andersson, M. 1994 *Sexual selection*. Princeton University Press.

Arnqvist, G. 1998 Comparative evidence for the evolution of genitalia by sexual selection. *Nature* **393**, 784–786.

Arnqvist, G., Edvardsson, M., Friberg, U. & Nilsson, T. 2000 Sexual conflict promotes speciation in insects. *Proc. Natl Acad. Sci. USA* **97**, 10 460–10 464.

Barraclough, T. G., Harvey, P. H. & Nee, S. 1995 Sexual selection and taxonomic diversity in passerine birds. *Proc. R. Soc. Lond.* B **259**, 211–215.

Bateman, A. J. 1948 Intrasexual selection in *Drosophila*. *Heredity* **2**, 349–368.

Birkhead, T. R. & Møller, A. P. 1998 *Sperm competition and sexual selection*. London: Academic.

Chapman, T., Liddle, L. F., Kalb, J. M., Wolfner, M. F. & Partridge, L. 1995 Cost of mating in *Drosophila melanogaster* is mediated by male accessory-gland products. *Nature* **373**, 241–244.

Clutton-Brock, T. H. & Parker, G. A. 1992 Potential reproductive rates and the operation of sexual selection. *Q. Rev. Biol.* **67**, 437–456.

Clutton-Brock, T. H. & Parker, G. A. 1995 Sexual coercion in animal societies. *Anim. Behav.* **49**, 1345–1365.

Corbet, A. S. & Pendlebury, H. M. 1992 *The butterflies of the Malay Peninsula*. Kuala Lumpar: Malayan Nature Society.

Corbet, G. B. & Hill, J. E. 1991 *A world list of mammalian species*, 3rd edn. Oxford: British Museum of Natural History. London & Oxford University Press.

Darwin, C. 1871 *The descent of man and selection in relation to sex*. London: Murray.

DeVries, P. J. 1987 *The butterflies of Costa Rica and their natural history, Papilionidae, Pieridae, Nymphalidae*. Princeton University Press.

Drummond, B. A. 1984 Multiple mating and sperm competition in the Lepidoptera. In *Sperm competition and the evolution of animal mating systems* (ed. R. L. Smith), pp. 291–370. London: Academic.

Eberhard, W. G. 1985 *Sexual selection and animal genitalia*. London: Harvard University Press.

Eberhard, W. G. 1996 *Female control: sexual selection by cryptic female choice*. Princeton University Press.

Elgar, M. A. 1998 Sexual selection and sperm competition in arachnids. In *Sperm competition and sexual selection* (ed. T. R. Birkhead & A. P. Møller), pp. 307–339. London: Academic.

Emlen, S. T. & Oring, L. W. 1977 Ecology, sexual selection and the evolution of mating systems. *Science* **197**, 215–223.

Gavrilets, S. 2000 Rapid evolution of reproductive barriers driven by sexual conflict. *Nature* **403**, 886–889.

Gavrilets, S., Arnqvist, G. & Friberg, U. 2001 The evolution of female mate choice by sexual conflict. *Proc. R. Soc. Lond.* B **268**, 531–539. (DOI 10.1098/rspb.2000.1382.)

Ginsberg, J. R. & Rubenstein, D. J. 1990 Sperm competition and variation in zebra mating behaviour. *Behav. Ecol. Sociobiol.* **26**, 427–434.

Gwynne, D. T. & Simmons, L. W. 1990 Experimental reversal of courtship roles in an insect. *Nature* **346**, 172–174.

Harcourt, A. H. 1991 Sperm competition and the evolution of non-fertilizing sperm in mammals. *Evolution* **45**, 314–328.

Harvey, P. H. & Pagel, M. D. 1991 *The comparative method in evolutionary biology*. Oxford University Press.

Hewitt, G. M., Mason, P. & Nichols, R. A. 1989 Sperm precedence and homogamy across a hybrid zone in the alpine grasshopper *Podisma pedestris*. *Heredity* **62**, 343–354.

Higashi, M., Takimoto, G. & Yamamura, N. 1999 Sympatric speciation by sexual selection. *Nature* **402**, 523–526.

Holland, B. & Rice, W. R. 1999 Experimental removal of sexual selection reverses intersexual antagonistic coevolution and removes a reproductive load. *Proc. Natl Acad. Sci. USA* **96**, 5083–5088.

Hosken, D. J. 1997 Sperm competition in bats. *Proc. R. Soc. Lond.* B **264**, 385–392. (DOI 10.1098/rspb.1997.0055.)

Hosken, D. J. 1998 Testes mass in megachiropteran bats varies in relation to sperm competition theory. *Behav. Ecol. Sociobiol.* **44**, 169–177.

Houde, A. E. & Endler, J. A. 1990 Correlated evolution of female mating preferences and male color patterns in the guppy *Poecilia reticulata*. *Science* **248**, 1405–1408.

Jiggins, C. D., Naisbit, R. E., Coe, R. L. & Mallet, J. 2001 Reproductive isolation caused by colour pattern mimicry. *Nature* **411**, 302–305.

Kenagy, G. J. & Trombulak, S. C. 1986 Size and function of mammalian testes in relation to body size. *J. Mammal.* **67**, 1–22.

Lande, R. 1980 Sexual dimorphism, sexual selection, and adaptation in polygenic characters. *Evolution* **34**, 292–305.

Lande, R. 1981 Models of speciation by sexual selection on polygenic traits. *Proc. Natl Acad. Sci. USA* **78**, 3721–3725.

Larsen, T. B. 1991 *The butterflies of Kenya and their natural history*. Oxford University Press.

Lindenfors, P. & Tullberg, B. S. 1998 Phylogenetic analyses of primate size evolution: the consequences of sexual selection. *Biol. J. Linn. Soc.* **64**, 413–447.

McMillan, W. O., Jiggins, C. D. & Mallet, J. 1997 What initiates speciation in passion-vine butterflies? *Proc. Natl Acad. Sci. USA* **94**, 8628–8633.

Magurran, A. E. 1998 Population differentiation without speciation. *Phil. Trans. R. Soc. Lond.* B **353**, 275–286. (DOI 10.1098/rstb.1998.0209.)

Mayr, E. 1963 *Animal species and evolution*. Cambridge, MA: Harvard University Press.

Morrow, E. H. & Gage, M. J. G. 2000 The evolution of sperm length in moths. *Proc. R. Soc. Lond.* B **267**, 307–313. (DOI 10.1098/rspb.2000.1001.)

Novacek, M. J. 1992 Mammalian phylogeny: shaking the tree. *Nature* **356**, 121–125.

Nylin, S., Nyblom, K., Ronquist, F., Janz, N., Belicek, J. & Källersjö, K. 2001 Phylogeny of *Polygonia, Nymphalis* and related butterflies (Lepidoptera: Nymphalidae): a total-evidence analysis. *Zool. J. Linn. Soc.* **132**, 441–468.

Owens, I. P. F., Bennett, P. M. & Harvey, P. H. 1999 Species richness among birds: body size, life history, sexual selection or ecology? *Proc. R. Soc. Lond.* B **266**, 933–939. (DOI 10.1098/rspb.1999.0726.)

Parker, G. A. 1970 Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**, 525–567.

Parker, G. A. 1979 Sexual selection and sexual conflict. In *Sexual selection and reproductive competition in insects* (ed. M. S. Blum & N. A. Blum), pp. 123–166. New York: Academic.

Parker, G. A. 1983 Arms races in evolution: an ESS to the opponent-independent costs game. *J. Theor. Biol.* **101**, 619–648.

Parker, G. A. & Partridge, L. 1998 Sexual conflict and speciation. *Phil. Trans. R. Soc. Lond.* B **353**, 261–274. (DOI 10.1098/rstb.1998.0208.)

Parker, G. A., Ball, M. A., Stockley, P. & Gage, M. J. G. 1997 Sperm competition games: a prospective analysis of risk assessment. *Proc. R. Soc. Lond.* B **263**, 1793–1802. (DOI 10.1098/rspb.1997.0249.)

Parsons, M. 1999 *The butterflies of Papua New Guinea: their systematics and biology*. San Diego, CA: Academic.

Purvis, A. & Rambaut, A. 1994 *Comparative analysis by independent contrasts (CAIC), v. 2.* Oxford University Press.

Rice, W. R. & Holland, B. 1997 The enemies within: intergenomic conflict, interlocus contest evolution (ICE), and the intraspecific Red Queen. *Behav. Ecol. Sociobiol.* **41**, 1–10.

Roberts, M. J. 1996 *Spiders of Britain and Northern Europe*. London: HarperCollins.

Samu, F., Toft, S. & Kiss, B. 1999 Factors influencing cannibalism in the wolf spider *Pardosa agrestis* (Araneae, Lycosidae). *Behav. Ecol. Sociobiol.* **45**, 349–354.

Scott, J. A. 1986 *The butterflies of North America*. Stanford University Press.

Seehausen, O. & von Alphen, J. M. 1999 Can sympatric speciation by disruptive sexual selection explain rapid evolution of cichlid diversity in Lake Victoria? *Ecol. Lett.* **2**, 262–271.

Short, R. V. 1997 The testis: the witness of the mating system, the site of mutation and the engine of desire. *Acta Paediatr.* **86**, 3–7.

Silva, M. & Downing, J. A. 1995 *CRC handbook of mammalian body masses*. Boca Raton, FL: CRC Press.

Simmons, L. W., Stockley, P., Jackson, R. L. & Parker, G. A. 1996 Sperm competition or sperm selection: no evidence for female influence over paternity in yellow dung flies *Scatophaga stercoraria*. *Behav. Ecol. Sociobiol.* **38**, 199–206.

Smart, P. 1975 *The illustrated encyclopedia of the butterfly world*. London: Salamander Books.

Smuts, B. B. & Smuts, R. W. 1993 Male aggression and sexual coercion of females in non-human primates and other mammals: evidence and theoretical implications. *Adv. Stud. Behav.* **22**, 1–63.

Spencer Smith, D., Miller, L. D. & Miller, J. Y. 1994 *The butterflies of the West Indies and South Florida*. Oxford University Press.

Stearns, S. C. 1983 The influence of size and phylogeny on patterns of covariation among life-history traits in mammals. *Oikos* **41**, 173–187.

Stockley, P. 1997 Sexual conflict resulting from adaptations to sperm competition. *Trends Ecol. Evol.* **12**, 154–159.

Svärd, L. & Wiklund, C. 1989 Mass and production rates of ejaculates in relation to monandry–polyandry in butterflies. *Behav. Ecol. Sociobiol.* **24**, 395–402.

Tregenza, T. & Wedell, N. 2002 Polyandrous females avoid costs of inbreeding. *Nature* **415**, 71–73.

Trivers, R. L. 1972 Parental investment and sexual selection. In *Sexual selection and the descent of man, 1871–1971* (ed. B. Campbell), pp. 136–179. London: Heinemann.

Turner, G. F. & Burrows, M. T. 1995 A model of sympatric speciation by sexual selection. *Proc. R. Soc. Lond.* B **260**, 287–292.

Tuzov, V. K. 1997 *Guide to the butterflies of Russia and adjacent territories*, vol. 1. *Hesperiidae, Papilonidae, Pieridae, Satyridae.* Sofia, Bulgaria: Pensoft.

Tuzov, V. K. 2000 *Guide to the butterflies of Russia and adjacent territories*, vol. 2. *Libytheidae, Danaidae, Nymphalidae, Riodinidae, Lycaenidae.* Sofia, Bulgaria: Pensoft.

van Doorn, G. S., Noest, A. J. & Hogeweg, P. 1998 Sympatric speciation and extinction driven by environment dependent sexual selection. *Proc. R. Soc. Lond.* B **265**, 1915–1919. (DOI 10.1098/rspb.1998.0520.)

Wahlberg, N. 2000 The ecology and evolution of Melitaeine butterflies. PhD thesis, University of Helsinki, Finland.

Wiklund, C. & Forsberg, J. 1991 Sexual size dimorphism in relation to female polygamy and protandry in butterflies: a comparative study of Swedish Pieridae and Satyridae. *Oikos* **60**, 373–381.

Wiklund, C., Kaitala, A. & Wedell, N. 1998 Decoupling of reproductive rates and parental expenditure in a polyandrous butterfly. *Behav. Ecol.* **9**, 20–25.