SEX-RATIO EVOLUTION IN SEX CHANGING ANIMALS

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Abstract.—Sex allocation theory is often able to make clear predictions about when individuals should facultatively adjust their offspring sex ratio (proportion male) in response to local conditions, but not the consequences for the overall population sex ratio. A notable exception to this is in sex changing organisms, where theory predicts that: (1) organisms should have a sex ratio biased toward the ''first'' sex; (2) the bias should be less extreme in partially sex changing organisms, where a proportion of the ''second'' sex matures directly from the juvenile stage; and (3) the sex ratio should be more biased in protogynous (female first) than in protandrous (male first) species. We tested these predictions with a comparative study using data from 121 sex changing animal species spanning five phyla, covering fish, arthropods, echinoderms, molluscs, and annelid worms. We found support for the first and third predictions across all species. The second prediction was supported within the protogynous species (mainly fish), but not the protandrous species (mainly invertebrates).

Key words.—Comparative analysis, protandrous, protogynous, sequential hermaphrodite, sex allocation, sex ratio.

Sex allocation theory describes how organisms should divide their resources between male and female reproduction (Charnov 1982). Some of the most striking successes of sex allocation theory have been in explaining cases in which individuals facultatively adjust their offspring sex ratios (proportion male) in response to local conditions (Charnov 1982; Hardy 2002; West et al. 2002a), as originally suggested by Trivers and Willard (1973). For example, numerous parasitic wasps have been shown to lay male eggs on relatively small hosts and female eggs on large hosts, because female offspring gain a greater fitness benefit from extra resources and larger body size (West and Sheldon 2002). In contrast, when such facultative sex ratio adjustment occurs, sex allocation theory has been much less successful in predicting and explaining variation in the overall population or breeding sex ratio (West et al. 2002a). The reason for this is that the population sex ratio is often predicted to depend upon biological details that are rarely known, such as the details of male and female life histories, and whether other behaviors such as clutch size are also facultatively adjusted (Frank 1987, 1990; Pen and Weissing 2000, 2002; West and Sheldon 2002).

Here we consider a case in which it is possible to make clear predictions for the overall population sex ratio. We are concerned with species in which sex change occurs, also termed sex reversal or sequential hermaphroditism. This has been documented in a variety of fish, invertebrates, and plants (Charnov 1982; Policansky 1982; Allsop and West 2003a). Sex allocation theory suggests that sex change is favored when the reproductive value of an individual varies with age or size, and the relationship is different for males and females. In this case natural selection favors individuals who mature as the sex whose reproductive value increases more slowly with age (first sex), and then change to the other sex (second sex) when older (Ghiselin 1969; Leigh et al. 1976; Charnov 1982; Warner 1988a,b).

Sex allocation theory is able to make three predictions for the overall breeding sex ratio in species where sex change occurs. First, the sex ratio should be biased toward the sex that individuals mature as, termed the first sex (Charnov 1982; Frank and Swingland 1988; Charnov and Bull 1989; Charnov 1993). Second, the sex ratio is predicted to show less extreme biases in partially sex changing species in which some individuals mature as the second sex (Charnov 1989). Third, the sex ratio should be more biased in protogynous (female first) than in protandrous (male first) species (Charnov 1982). Previous work testing these predictions has been largely anecdotal, and relied primarily upon only a few species from a limited number of taxa, such as a comparison of female first (protogynous) fish with male first (protandrous) shrimps (Charnov 1982, 1993; Charnov and Bull 1989). Comparisons between limited numbers of taxa can be a problem because other phylogenetically conserved differences can lead to misleading correlations (Harvey and Pagel 1991). Closely related species tend to share many characters through common descent rather than independent evolution; thus differences in the sex ratios of fish and shrimps, for example, could be explained by other factors that also differ between them.

In this study we carry out a quantitative test of these three predictions for the population sex ratios of sex changing species. We expand upon previous work in two ways. First, we use a taxonomically comprehensive dataset with 121 animal species, spanning a wide range of taxa, including fish, crustaceans, molluscs, echinoderms, and annelid worms. Second, we carry out the first phylogenetically based comparative tests of the theoretical predictions. We construct a ''supertree'' of relationships for sex changing animals combining information from a wide variety of published sources that use both taxonomic and phylogenetic information. We map the direction of sex change and presence of early maturers onto this tree, which allows us to test the predictions of sex allocation theory using the comparative methodology of independent contrasts (Felsenstein 1985). Before describing our work, we explain the theoretical predictions that we are testing.

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Sex Change Theory

First, why is the sex ratio predicted to be biased toward the sex that individuals mature as first (Charnov 1982, 1993; Frank and Swingland 1988; Charnov and Bull 1989; Charnov and Skuladottir 2000)? This can be explained following Charnov (1993). Consider the case of a protogynous diploid species, in which individuals mature as females and then change sex to males when older (bigger). In this case the relative fitness of males increases faster with age than it does for females. Males and females must make an equal genetic contribution to the next generation, because all offspring have two parents. Consequently, it must be true that

$$N_m W_m = N_f W_f, \tag{1}$$

where N_m and N_f are the number of mature males and females and W_m and W_f represent the reproductive value (fitness) of a male and a female. Given that the reproductive value of a male at the point of sex change will be equal to that of a female, and that male reproductive value increases faster with age, it follows that $W_m > W_f$, because any individual that has become a male must have a higher fitness than individuals that are still female. Consequently, for equation (1) to hold, it also follows that $N_m < N_f$. This means that there will be more females than males, and hence a female-biased sex ratio. The converse prediction for protandrous (male first) species can equally be made, showing that a male-biased sex ratio is predicted.

Second, why should the sex ratio be less biased if early maturers of the second sex are present (Charnov 1989)? Consider a protogynous population (changes sex from female to male) with a proportion of males arising directly from the juvenile state without prior transition through the female phase (termed early maturers or pure males). The males in the population will therefore be divided between early maturing males and those matured as females, but then changed sex to male, termed terminal phase males.

On average, terminal phase males will have a higher reproductive value than early maturing males, mating with more females per unit time. This must be the case because sex change is only stable if male reproductive value increases substantially with age (Ghiselin 1969; Charnov 1982; Warner 1988a,b). Because early maturing males have a lower reproductive value than terminal phase males, the presence of early maturing males lowers the average reproductive value of males (W_m). Consequently, a lower ratio of N_f/N_m will be required to satisfy equation (1), leading to a less female-biased sex ratio. The converse prediction for protandrous (male first) species can equally be made, with the presence of early maturing females leading to a less male-biased sex ratio.

Third, why should protogynous (female first) species have more biased sex ratios than protandrous (male first) species (Charnov 1982)? In invertebrates and fish, female fecundity almost always increases rapidly with size, often following a cubed relationship (Charnov 1982, 1993). This means that differences in the direction of sex change are determined primarily by variation in how male fitness varies with size: in protogynous species, male fitness must increase even more rapidly with size, whereas in protandrous species, male fitness increases little or not at all with larger body size (Charnov 1982; Warner 1984). Consequently, if we assume that the fitness of individuals at the size of sex change is 1.0, then in protandrous species the average W_m will be approximately or just less than 1.0, and W_f will be much greater than 1.0. In contrast, with protogynous species, the average W_m will be much greater than 1.0 and the average W_f will be much lower than 1.0. This leads to a greater difference between W_m and W_f in protogynous species; thus, according to equation (1), a more biased sex ratio.

METHODS

Data Collection

We gathered sex ratio data for sex changing animals by: (1) performing ISI Web of Science database searches using keywords ''sex change'' + ''sex ratio''; (2) searching in key reviews on the topic (Reinboth 1975; Robertson and Warner 1978; Warner and Robertson 1978; Policansky 1982; Charnov and Bull 1989); (3) searching citations in all papers found; (4) directly from the field, for the marine goby *Coryphopterus personatus*, as part of a larger study (Allsop and West 2004); and (5) using data from our previous comparative studies on life-history variables in sex changing organisms (Allsop and West 2003a,b). In some studies, authors determined sex by macroscopic observation of the genitalia or secondary sexual characters (Cole 1983; Abe and Fukuhara 1996). In others, sex was determined by histological examination of the gonads (Gillanders 1995; Brule et al. 1999). In all cases, we have only included data for the sexually mature members of any populations sampled; thus the analysis does not include juveniles, as assumed by theory.

For the purposes of investigating the impact of mixed populations (sex changers and early maturers together) on the sex ratio, we carried out the analysis in two ways. We first followed (Charnov 1989) by categorizing a species as a pure sex changer (not mixed) if the proportion of early maturing males or females (animals of the second sex in the initial phase) was less than 2%. However, the proportion of primary individuals has been quantified in only a limited number of species, mainly protogynous fish. Consequently, to examine this question more generally, we also carried out separate analyses, assigning mixed or pure sex changing status based upon the authors' description of the organisms' life history—specifically, whether the presence of any early maturing individuals had been noted. For example, Pollock's study on the protandrous yellowfin bream, *Acanthopagrus australis* (Pollock 1985, p. 301), states that ''..most juveniles become functional males by the age of two years but a small proportion of juveniles develop directly into functional females (primary females).'' Although Pollock goes on to suggest the possible developmental origin of these small females, he does not at any point quantify their proportion in the population. In cases such as this we assign the species as being mixed (having early maturers of the second sex), for our second analysis. Alternatively, if a study presents sex/size frequency data, and highlights that there is no overlap between the large males (females) and small females (males), such as is discussed in Lowry and Stoddart (1986) for populations of the amphipod *Acontiostoma marionis*, we assigned such species

as being pure sex changers (having no early maturers). Although it is certainly possible that other populations of these species might show plasticity in the production of early maturers in different locations, we believe the assignment of mixed or pure status using these criteria for the populations under study here is appropriate for the current analysis.

We excluded data for the anemone fish (Amphiprioninae) from all analyses, since the sex ratio has been argued to be extrinsically constrained to 0.5. This is because the size and spatial distribution of their host anemones necessitates the formation of monogamous pairs (Fricke and Fricke 1977). This is analogous to the situation in apicomplexan (protozoa) parasites, where syzygy leads to a form of monogamy and selection for an unbiased sex ratio even with high levels of inbreeding (West et al. 2000, 2002b).

Phylogenetic Relationships

We obtained relationships within the invertebrates from the following sources: the Crustaceans from Brook et al. (1994) and Tsai et al. (1999); the Echinoderms from Sewell (1994); and relationships amongst the other invertebrate taxa from the Tree of Life web project (http://tolweb.org/tree/phylogeny.html), since we were unable to locate recent published records of relationships. These invertebrate phylogenies are based primarily upon morphological characters.

For the phylogenetic relationships within fish, in most cases we were able to use recently published information. Specifically, relationships for the Pomacentridae were inferred from Godwin (1995), based upon morphology and biogeography; Lethrinidae were obtained from Lo Galbo et al. (2002), who generated a molecular phylogeny using the cytochrome *b* gene; Scaridae relations were taken from Streelman et al. (2002), who used nuclear and mitochondrial DNA genes; Labridae were obtained from Hanel et al. (2002) and M. W. Westneat (pers. comm. 2002), based upon morphological characters as well as mitochondrial and nuclear DNA; and Sparidae relations were inferred from Hanel and Sturmbauer (2000), Orrell et al. (2002), Desdevises et al. (2002) and from Hanel (pers. comm. 2003). In cases where published information was not available, and for higher-level relationships, we used Nelson (1994).

Although our tree of relationships is constructed from a combination of taxonomic and phylogenetic information, and hence suffers from the problem of housing paraphyletic groups (Starck 1998), such as invertebrates, crustacea, and fish, it is the first effort that we know of to comprehensively investigate the evolutionary pathways of the sex change life-history strategy. As such it allows the best current effort to investigate the evolution of the sex ratio in sex changers while controlling for the confounding effects of evolutionary relatedness.

Statistical Analyses

We analyzed our data using two methods: assuming species were independent data points (SI), and independent contrasts (IC). The pros and cons of different comparative methods have been much debated, and a recent review focused on sex allocation is provided by Mayhew and Pen (2002). We used two methods because: (1) the theoretical predictions are in

some cases for specific values of the sex ratio (i.e. $>$ or $<$ 0.5)—this can only be assessed with SI, because IC test for correlations and relative differences, not specific values (for further discussion on this point see West et al. 2000); (2) this is necessary to show how differences with previous conclusions (Charnov 1989, Charnov and Bull 1989) arise due to either our expanded dataset or methods of analysis; (3) we wish to test how robust our results are to different forms of analysis; (4) differences and similarities between the conclusions drawn from these different analyses can be very informative (reviewed by Mayhew and Pen 2002); for example, they can show the extent of phylogenetic effects (Pagel 1993), and whereas IC examines evolutionary correlations, SI examines trends in extant characters that are the product of these evolutionary correlations (Mayhew and Pen 2002). More generally, studies of the sex ratio have provided some of the best examples of the adaptive process in action, often with staggering fits between theoretical predictions and empirical data (West and Herre 2002), and considerable inroads have been made toward defining the problem in a phylogenetic context (Herre et al. 2001; Mayhew and Pen 2002). However, ours is the first such study to address the evolution of the sex ratio in sex changing organisms using a phylogenetically based comparative method.

We first analyzed the data assuming that species were independent datapoints. We are interested in the sex ratio, defined as the proportion of individuals that are male. Proportion data such as sex ratio usually have nonnormally distributed error variance and unequal sample sizes. To avoid these problems, we analyzed the data with a generalized linear model analysis of deviance, assuming binomial errors, and a logit link function (Crawley 1993; Wilson and Hardy 2002). However, the data were highly overdispersed, with the residual deviance 174 to 252 times the residual degrees of freedom (this ratio is the heterogeneity factor), suggesting that a binomial error structure was not appropriate. Consequently, we arcsine transformed the sex ratio data, confirmed that the error variance was normally distributed using the Kolmogorov-Smirnov test for normality ($\chi^2 = 1.34$, $P > 0.1$), and carried out our analysis using standard ANOVA and ANCOVA. All mean sex ratio results presented are back transformed. We tested the extent of sex ratio deviation from 0.5 by calculating a mean magnitude of deviation (MMD) for each species (i.e. for protandrous species, MMD = sex ratio -0.5; for protogynous species, MMD = $0.5 -$ sex ratio). The sex ratio deviation data fit the assumption of normal error distribution without the need for any further transformation (Kolmogorov-Smirnov test for normality: $\chi^2 = 2.4$, $P > 0.1$). We investigated the effects of pure and mixed populations on the sex ratio in three ways: performing ANOVA using pure/mixed status assigned using the two methods above as a dichotomous predictor, and by treating the proportion of early maturers as a continuous predictor.

We also analyzed our data using a phylogenetically based comparative method. There are a variety of different statistical methods for removing the effects of evolutionary relatedness from cross-species analyses. These include the phylogenetic generalized least-squares method (Grafen 1989), the method of independent contrasts (Felsenstein 1985), the phylogenetic eigenvector regression (Diniz-Filho et al. 1998)

and the autoregressive method (Cheverud and Dow 1985). Although the debate over which method is the best to use is still active (Rohlf 2001), we chose to use the most commonly applied technique, the method of independent contrasts (Felsenstein 1985), as implemented in the CAIC statistical package (Purvis and Rambaut 1995). Independent contrasts (ICs) are derived by calculating the difference in the response and the explanatory variables across pairs of species, or higher nodes that share a common ancestor. The CAIC package uses the method of Pagel (1992) for continuous variables and Burt (1989) for dichotomous variables. We tested for significance in the dichotomous analyses using a one-sample t-test, comparing the mean of the standardized contrasts to zero. In all cases, unless otherwise stated, the data fit the assumption of normality (Mayhew and Pen 2002). We assigned branch lengths using both the Grafen (1989) and Pagel (1992) methods—in all cases these gave the same result, and so we have only reported analyses using the Pagel method. The species relationships that we used for the independent contrasts analysis are given in Figure 1.

RESULTS AND DISCUSSION

Our dataset represents 196 separate populations from 121 species spanning 26 families from five phyla (Annelida, Mollusca, Arthropoda, Echinodermata, and Chordata; see Fig. 1. and the Appendix available online at http://dx.doi.org/10.1554/03-602.1.s1). Broadly speaking, we find protogynous (female to male) sex change within the vertebrates, and protandrous sex change in the invertebrate taxa. Notable exceptions to these generalities are as follows. Within the vertebrates (which are all fish) the families Sparidae (porgies) and Pomacentridae (damselfishes) have both protandry and protogyny; the sole representative from the family Polynemidae (threadfins) is protandrous; the Gobiidae (gobies) and Cirrhitidae (hawkfishes; no members of the Cirrhitidae family are represented in the current analysis) are now known to have species capable of switching back and forth between the sexes (both-ways sex change); and the Clupeidae (herring, sardines, etc.) are protandrous. Within the invertebrates the Peracaridan crustaceans (containing the isopods, amphipods, and tanaidaceans) have both protogynous and protandrous species; within the order Isopoda (wood lice and relatives) there are both protandrous and protogynous species; and the sex changers in the crustacean order Tanaidacea are all protogynous. Where information on the presence or absence of early maturers was available, there appeared to be no clear pattern for its distribution throughout the taxonomic groups, with both mixed and pure populations existing in all major phyla except for the Mollusca, which appear to have no recorded instances of early maturing individuals of the second sex.

Direction of Sex Change and the Sex Ratio

We first tested the prediction that the sex ratio should be biased towards the first sex (Charnov 1982, 1993; Frank and Swingland 1988; Charnov and Bull 1989; Frank 1998). All our analyses supported this prediction. Assuming species as independent datapoints: (1) the sex ratio of protogynous (female first) animals is significantly female biased (one-sample t-test comparing sex ratio to 0.5: $t = 4.1$, df $= 72$, $P < 0.001$), with a mean of 0.32 (SE $= 0.02$, $n = 73$); (2) the sex ratio of protandrous (male first) species were significantly male biased ($t = 10.9$, df $= 39$, $P < 0.001$), with a mean of 0.57 (SE $= 0.03$, $n = 40$; Fig. 2). This difference between groups is highly significant, with the sex ratio of protogynous species being more female biased than that of protandrous species ($F_{1,111} = 59.4$, $P < 0.001$). Overall, 96% of protogynous species had a female-biased sex ratio and 70% of protandrous species had a male-biased sex ratio (Fig. 2).

This result was confirmed by a comparative analysis with independent contrasts. In this case the hypothesis under test is that the sex ratio of protogynous taxa should be more female biased than the sex ratio of protandrous sister taxa. Our phylogeny of sex changing animals reduced to four independent contrasts in which the direction of sex change differs between sister taxa (Fig. 1). As predicted, in all cases the species that change sex from female to male were more female biased than the sister taxa that changed from male to female (Fig. 3). This difference was statistically significant, with the mean of the standardized contrasts being below zero ($t = -4.3$, df $= 3$, $P < 0.05$, $n = 4$ ICs).

Early Maturers and the Sex Ratio

We then tested the prediction that the sex ratio should be less biased in partially sex changing species in which a proportion of individuals mature directly into the second sex (Charnov 1989). We found support for this in the protogynous (female first) species, but not in the protandrous (male first) species (Figs. 2 and 4).

Considering protogynous fish, sex ratios were less female biased in species with early maturing males. This result held when using species as datapoints (using 2% rule: species with early maturers mean magnitude of deviation from a sex ratio of 0.5 (EM MMD) $= 0.12$, SE $= 0.02$, $n = 20$; pure sex changers mean magnitude of deviation (Pure MMD) $= 0.22$, SE $= 0.03$, $n = 20$; $F_{1,38} = 6.27$, $P < 0.05$; relaxing 2% rule: EM MMD $= 0.14$, SE $= 0.02$, $n = 30$; Pure MMD $= 0.21$, SE $= 0.03$, $n = 26$; $F_{1,54} = 4.4$, $P < 0.05$), or when using ICs (using 2% rule: only three contrasts, thus no test; relaxing 2% rule: $t = 2.9$, df $= 8$, $P < 0.05$, $n = 9$ ICs). In the fish species where the proportion of early maturers had been quantified, we also tested whether the variation in the proportion of early maturers could be used as a continuous predictor of the sex ratio. In this case there was no significant correlation between the sex ratio and the proportion of early maturing males, irrespective of whether species ($F_{1,41} = 0.68$, $P > 0.1$, $r^2 = 0.02$, $n = 43$) or independent contrasts ($F_{1,19} = 0.64$, $P > 0.1$, $r^2 = 0.03$, $n = 19$ ICs) were used as datapoints.

Examining all protogynous species (vertebrates and invertebrates together), there was no significant trend for species with early maturers to have a less biased sex ratio when data were analyzed with species as datapoints (2% rule relaxed: EM MMD $= 0.16$, SE $= 0.02$, $n = 33$; Pure MMD $= 0.21$, SE $= 0.03$, $n = 27$; $F_{1,58} = 0.08$, $P > 0.1$), but there was a significant effect with the method of independent contrasts ($t = 2.3$, df $= 10$, $P < 0.05$, $n = 11$ ICs; Fig. 4). This difference of results between the IC and the SI methods of
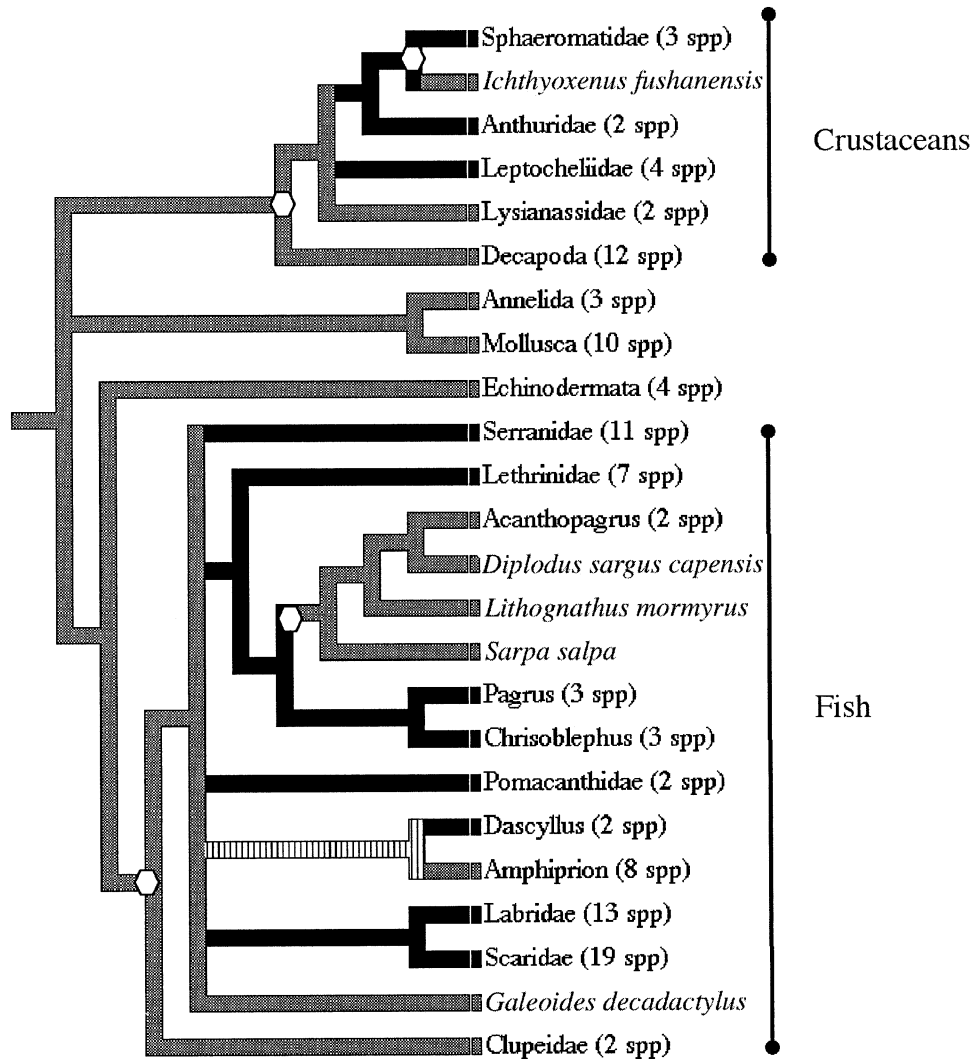
FIG. 1. A composite phylogeny for sex changing animals. Black, female first (protogynous); gray, male first (protandrous); vertical lines, equivocal. Branch lengths do not represent evolutionary time. Independent contrasts for the direction of sex change are marked as white hexagons.

analysis reflects the fact that sister taxa that differ in the presence or absence of early maturers do have significant differences in the magnitude of sex ratio bias, but these differences are hidden in a simple SI analysis because there is so much variation in the magnitude of sex ratio bias when all taxonomic groups in the dataset are considered together. This provides a clear example of exactly the kind of problem that the IC method can deal with (Harvey and Pagel 1991). To our knowledge, this is the first time that such a discrepancy has been found in comparative work on sex allocation, as previous analyses have usually found qualitatively and quantitatively identical results with IC and SI analyses (Herre et al. 2001; Mayhew and Pen 2002).

Examining the protandrous species, there was no significant trend for species with early maturers to have a less biased sex ratio, when using species as datapoints (2% rule relaxed: EM MMD = 0.17, SE = 0.08, $n = 6$; Pure MMD = 0.08, SE = 0.04, $n = 18$; $F_{1,22} = 0.88$, $P > 0.1$). Indeed, the method of independent contrasts even hints at the trend being

reversed in protandrous animals, with mixed species having more biased sex ratios ($t = 4.8$, df = 2, $P < 0.05$, $n = 3$ ICs), although this is based upon only three ICs.

Consequently, our results support Charnov's (1989) prediction that species with early maturers should have less biased sex ratios when considering protogynous species (mainly fish), but not when considering protandrous species (mainly invertebrates). Furthermore, although protogynous species show a difference between species with and without early maturers, there was no significant effect of the proportion of early maturers. Possible explanations for these differences fall into three broad categories. First, the sex ratio is predicted to depend upon a number of factors (Charnov 1982, 1993; Charnov and Bull 1989; Frank and Swingland 1988; Frank 1998; Charnov and Skuladottir 2000), thus the effect of proportion of early maturers may be confounded by other variables. Analyzing the data with independent contrasts can get around this problem (Harvey and Pagel 1991; Mayhew and Pen 2002), as our analyses have shown, but in other cases

Protogynous Species
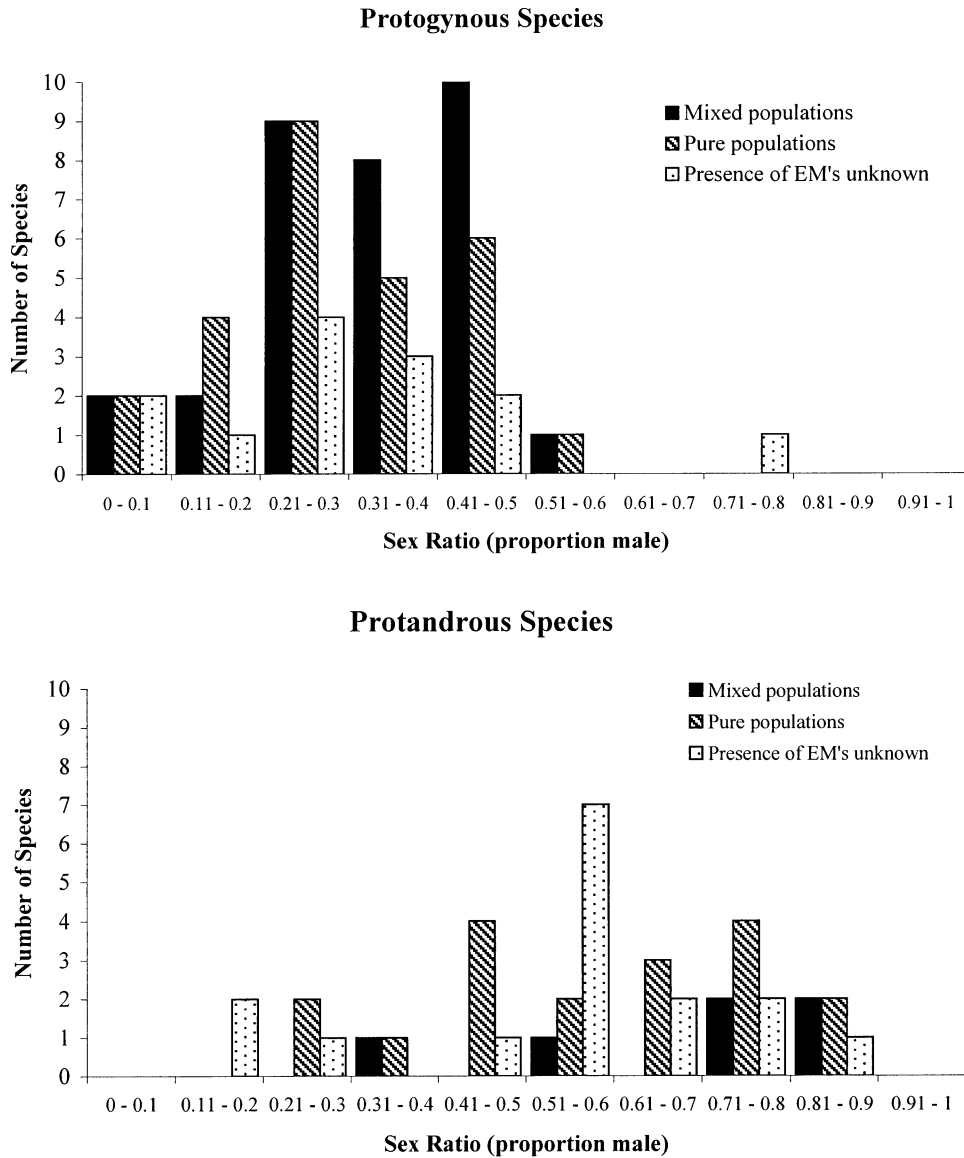

Protandrous Species

FIG. 2. Frequency distribution of population sex ratios for all protogynous and protandrous sex changers (excluding eight species of anemone fish—see Methods for details). Histograms are split between pure sex changing species (with no early maturers [EMs] of the second sex), mixed sex changing species (with a proportion of early maturers of the second sex) and species for which the presence or absence of early maturers is unknown.

limited phylogenetic resolution severely limited our statistical power (e.g., $n = 3$ ICs in some analyses). It would be extremely useful to create more resolved phylogenies for these species, as well as quantifying the proportion of early maturers in more species, and other factors that may influence the sex ratio. Second, the biology of the protandrous species may differ in important ways from the assumptions of theory. This seems particularly possible with the invertebrates, which have been relatively less well studied. For example: (1) in the Tanaid crustacean *Leptochelia dubia*, males are born with no mouthparts and thus suffer much higher mortality rates than females (Highsmith 1983), in contrast to the constant mortality assumption of theory (Charnov 1993); (2) in the flesh-burrowing parasitic isopod *Ichthyoxenus fushanensis*, which inhabits a membranous cavity in the body wall of the

freshwater fish *Varicorhinus bacbatulus* (Tsai et al. 1999), resource limitation may lead to a monogamous lifestyle that could constrain the sex ratio to be 0.5 as with anemone fish (see Methods). Third, there could be some sampling bias with the data collection, possibly due to factors such as the small size of one sex, or relatively extreme habitats. This possibility is illustrated by the fact that it has been possible to quantify the existence of early maturing individuals only rarely in some species (especially invertebrates), compared with others (some fish).

Sex Ratio Bias: Protogynous versus Protandrous Species

Finally, we tested Charnov's (1982) prediction that the sex ratio should be more biased in protogynous (male first) than
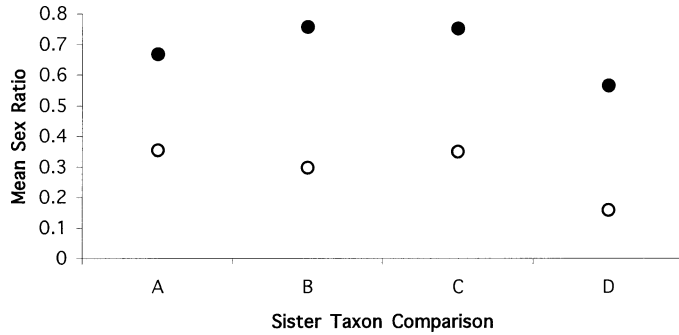
FIG. 3. Independent contrasts for mean sex ratios of sex changing taxa showing protandrous (male first; filled circles) and protogynous (female first; unfilled circles) animals separately. All protogynous animals have a sex ratio below that of the protandrous animals, as predicted by theory. Letters along the x-axis refer to the taxonomic groups being contrasted in our formal comparative analysis: A, Sparidae fish (five protandrous species, six protogynous species); B, Flabelliferan isopods (one protandrous, three protogynous); C, Teleost fish (other than those in the families Sparidae and Pomacentridae, for whom lower level contrasts are computed; three protandrous, 56 protogynous); D, Peracaridan crustaceans (other than those included in the lower level contrast described above in letter C; two protandrous species, six protogynous species).

in protandrous (female first) species. We found some, but not complete, support for this prediction. When considering species as datapoints, the mean magnitude of the deviation from 0.5 was significantly greater for protogynous species (MMD = 0.19, SE = 0.02, n = 73), than it was for protandrous species (MMD = 0.07, SE = 0.03, n = 40; $F_{1,111}$ = 13.1, $P < 0.001$). However, when using the independent contrasts method, we did not find statistical support for this result (t

= 2.13, df = 3, $P > 0.1$, n = 4 ICs), although our power to test this prediction is limited due to having only four independent contrasts. However, the data are in the predicted direction, with three of four ICs above zero. Clearly, increased phylogenetic resolution and targeted data collection would be extremely useful for expanding the number of IC's that could be used in this analysis, and hence determining if the same result will be obtained as with SI analyses. In particular, it would be extremely useful to construct molecular phylogenies of the invertebrate species, and to increase resolution within the Teleost fish order Perciformes, which is polyphyletic with poor phylogenetic resolution of the suborders (Lauder and Liem 1983; Asoh and Kasuya 2002).

Conclusions

We analyzed data on the adult sex ratio in 121 sex changing animal species from five phyla, covering fish, crustaceans, echinoderms, molluscs, and annelid worms (Fig. 1). We found support for the theoretical predictions that: (1) the sex ratio is biased toward the sex that individuals first reach reproductive maturity as (first sex) (Fig. 2 and 3); (2) the sex ratio is less biased in species in which there are some individuals who mature early as the second sex, when examining protogynous (female first) species (Fig. 2 and 4); (3) protogynous species show a greater deviation from a sex ratio of equality than protandrous (male first) species (Fig. 2). In contrast, we did not find support for the prediction that the sex ratio should be less biased in species in which there are some individuals who mature early as the second sex, when examining protandrous species. Possible explanations for this discrepancy with theory include multiple factors influencing
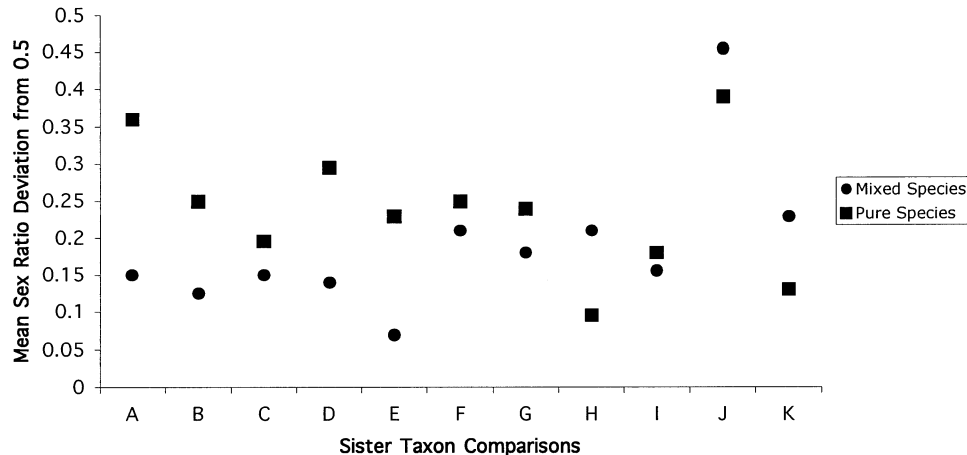

FIG. 4. Independent contrasts for the extent of sex ratio bias from 0.5 in all protogynous species (vertebrates and invertebrates), showing mixed species (those with early maturers of the second sex present; circles) against pure species (those with no early maturers present; squares). There is a significant trend for pure sex changing species to have a larger sex ratio deviation from 0.5 than species with early maturers. Letters along the x-axis refer to the taxanomic groups being contrasted in our formal comparative analysis: A, sub-branch of the Labridae fish family containing *Semicossyphus pulcher* and *Bodianus rufus* (one mixed taxa, one pure taxa); B, sub-branch of the Labridae fish family containing genus *Halichoerus* and species *Labroides dimidiatus* (one mixed taxa, one pure taxa); C, sub-branch of the Scaridae fish family containing the genus *Scarus* (10 mixed taxa, two pure taxa); D, sub-branch of the Scaridae fish family containing the genus *Sparisoma* (five mixed taxa, two pure taxa), E, sub-branch of the Serranidae fish family (multiple node) containing the genus *Epinephelus* (one mixed taxa, one pure taxa); F, sub-branch of the Serranidae fish family (multiple node) containing the genus *Epinephelus* (one mixed taxa, one pure taxa); G, sub-branch of the Serranidae fish family containing *Pseudoanthias squamipinnis* and *Plectropomus leopardus* (one mixed taxa, one pure taxa); H, the fish family Lethrinidae (one mixed taxa, eight pure taxa); I, the fish family Gobiidae (one mixed taxa, one pure taxa); J, the Peracaridan crustaceans *Hargeria rapax*, *Leptochelia forresti*, and *Leptochelia dubia* (two mixed taxa, one pure taxa); K, root of the tree (one mixed taxa, one pure taxa).

the sex ratio, theory not matching biology, and sampling bias. Furthermore, it suggests that there may be important differences between vertebrate and invertebrate species. Future progress in this area requires increasing phylogenetic resolution, quantification of further factors that may influence the sex ratio, and in particular more detailed species-specific studies of protandrous invertebrates.

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