



DISENTANGLING THE CONTRIBUTION OF SEXUAL SELECTION AND ECOLOGY TO THE EVOLUTION OF SIZE DIMORPHISM IN PINNIPEDS

Oliver Krüger,^{1,2} Jochen B. W. Wolf,³ Rudy M. Jonker,¹ Joseph I. Hoffman,¹ and Fritz Trillmich¹

¹Department of Animal Behaviour, Bielefeld University, PO Box 10 01 31, 33501 Bielefeld, Germany

²E-mail: oliver.krueger@uni-bielefeld.de

³Department of Evolutionary Biology, Evolutionary Biology Centre, Uppsala University, Norbyvägen 18D, 75236 Uppsala, Sweden

Received August 27, 2013

Accepted January 17, 2014

The positive relationship between sexual size dimorphism (SSD) and harem size across pinnipeds is often cited as a textbook example of sexual selection. It assumes that female aggregation selected for large male size via male–male competition. Yet, it is also conceivable that SSD evolved prior to polygyny due to ecological forces. We analyzed 11 life-history traits in 35 pinniped species to determine their coevolutionary dynamics and infer their most likely evolutionary trajectories contrasting these two hypotheses. We find support for SSD having evolved prior to changes in the mating system, either as a consequence of niche partitioning during aquatic foraging or in combination with sexual selection on males to enforce copulations on females. Only subsequently did polygyny evolve, leading to further coevolution as the strength of sexual selection intensified. Evolutionary sequence analyses suggest a polar origin of pinnipeds and indicate that SSD and polygyny are intrinsically linked to a suite of ecological and life-history traits. Overall, this study calls for the inclusion of ecological variables when studying sexual selection and argues for caution when assuming causality between coevolving traits. It provides novel insights into the role of sexual selection for the coevolutionary dynamics of SSD and mating system.

KEY WORDS: Comparative analysis, evolutionary pathway analysis, harem, mating system, seals.

EVOLUTION
INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

One of the most commonly observed features across the animal kingdom is that males and females differ in size, sometimes considerably (Fairbairn 1997; Székely et al. 2000; Fairbairn et al. 2007). The evolutionary and ecological significance of intersexual size differences has attracted attention since Darwin (1859) who coined the term *sexual selection* to explain the evolution of traits that increase mating success, but which may be maladaptive in terms of survival (Darwin 1871). Much cited examples of sexually selected traits are large male body size and secondary sexual characters such as elaborate weaponry that have evolved to facilitate intense male–male competition for access to females (Trivers 1972; Clutton-Brock 1991; Andersson 1994; Thomas et al. 2006). However, although such differences between the sexes have tradi-

tionally been interpreted in the light of sexual selection (Darwin 1871), they could also result from natural selection operating differently on the two sexes in respect of other aspects of a species' biology, such as resource partitioning (Shine 1989; Radford and du Plessis 2003; Ruckstuhl and Clutton-Brock 2005; Staniland 2005). Unfortunately, it can be difficult to disentangle such effects because sexual and natural selection may interact in complex ways (Andersson 1994; Krüger 2005; Krüger et al. 2007).

A classic example illustrating the power of sexual selection is the correlation between sexual size dimorphism (SSD) and the degree of polygyny in pinnipeds (seals, sea lions, and walruses; Bartholomew 1970; Lindenfors et al. 2002; Staniland 2005). In some species, males can be up to seven times heavier than

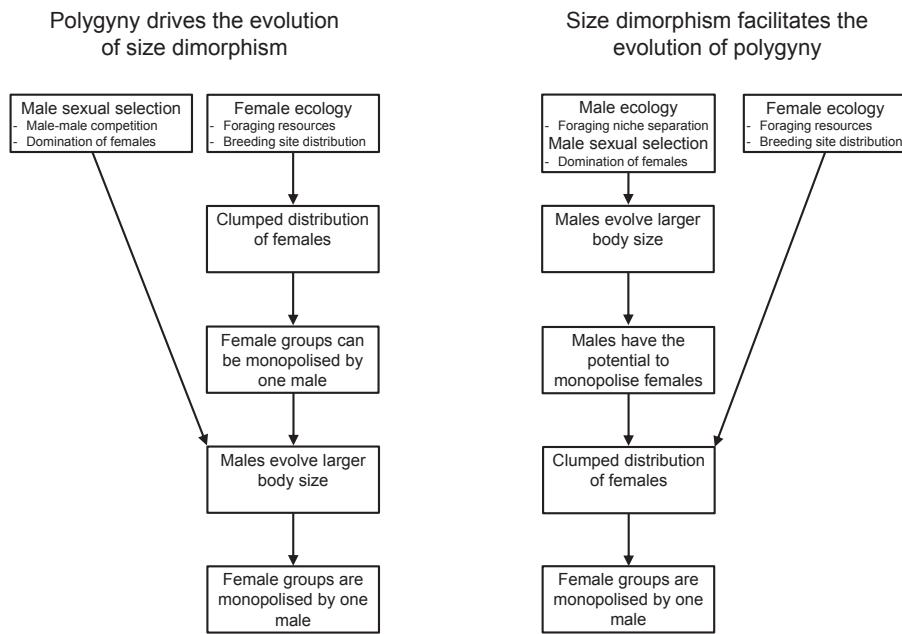


Figure 1. Schematic of two alternative hypotheses relating to the coevolution of polygyny and size dimorphism in pinnipeds.

females, with the most successful individuals monopolizing harems of over 50 females (Lindenfors et al. 2002). The interpretation of this positive relationship states that as soon as female groups exist, males are under progressively stronger selection to become large to remain competitive as male–male competition for access to female groups increases. The ensuing high reproductive skew among males leads to increased male harassment, which females avoid by grouping into progressively larger harems defended by a strong male. Hence, evolution proceeds through a positive coevolutionary feedback mechanism (Bartholomew 1970). This is consistent with recent findings that polygynous pinniped species have accelerated rates of evolution in sexually selected traits compared with promiscuous ones (Fitzpatrick et al. 2012b) and that directional selection has increased male body mass (Lindenfors et al. 2002; Fitzpatrick et al. 2012a).

Surprisingly, the causality of the relationship between SSD and mating system in pinnipeds has always been assumed rather than explicitly tested, despite both Fairbairn (1997) and especially Lindenfors et al. (2002) having pointed out that this “may not be completely straightforward and SSD could in theory come about independent of sexual selection.” One way of testing this causality indirectly is searching for a correlation between annual variance in male reproductive success and SSD. Gonzalez-Suarez and Cassini (2014) found no such correlation across eight seal species. It is hence not inconceivable that males could have evolved larger size prior to the evolution of polygyny, for example, due to foraging niche separation or to force copulations onto females (Cassini 1999). However, to discriminate between these possibilities requires an analytical framework that, unlike traditional comparative approaches, is robust to confounding variables

and allows the inference of causation (Fairbairn 1997; Perez-Barberia et al. 2002). Such a framework is provided by modern phylogenetic comparative approaches that have been used to estimate rates of evolution and reconstruct the most likely evolutionary pathways among multiple coevolving traits (Pagel 1999; Krüger and Davies 2002; Boerner and Krüger 2008; Krüger et al. 2009; Fitzpatrick et al. 2012a, b; von Hardenberg and Gonzalez-Voyer 2013).

Pinnipeds are uniquely suited to unravel the causal relationships between SSD and harem size. Although this monophyletic group comprises only 35 extant species (Berta and Churchill 2012), these vary greatly in both SSD and the degree of polygyny. Additionally, a wealth of information is available including ecological (Caro et al. 2012) and life-history (Trillmich 1996; Fitzpatrick et al. 2012a, b) variables that can be used to evaluate relative support for different evolutionary pathways. Here, we analyze these data to distinguish between two hypothetical pathways for the coevolution of SSD and mating system in pinnipeds (Fig. 1):

1. Polygynous mating systems promote directional selection on male body size, known to provide a direct advantage in intermale competition for access to females (Clutton-Brock 1988). Under this scenario, size dimorphism evolved as a direct consequence of evolutionary change in the mating system toward ever more extreme polygyny.
2. Alternatively, SSD evolved due to other forces of natural or sexual selection and only subsequently enabled males to herd and monopolize multiple females, thereby facilitating the evolution of polygyny as a consequence.

We evaluate support for these alternative scenarios and explore in detail how evolutionary changes in SSD and mating system were integrated into the evolutionary trajectory of other ecology and life-history parameters during pinniped evolution.

Methods

DATA COLLECTION

We collated available data on all 35 currently recognized, extant pinniped species from the literature (Lindenfors et al. 2002; Ferguson and Higdon 2006; Caro et al. 2012) including the following continuous variables: male body weight, female body weight, the resulting SSD (i.e., the ratio of male to female body weight), harem size, mean breeding latitude in degrees from the equator, the length of the breeding season in days, and the length of the lactation period in days. We also included dichotomous variables from Caro et al. (2012) such as whether a species breeds on ice or on land, if it is at risk of predation, whether copulation takes place in the water or on ice/land, whether the species makes shallow or deep dives, as well as female group size, and whether there is sexual dimorphism in pelage coloration. Data are summarized in Table S1.

PHYLOGENETIC COMPARATIVE ANALYSIS

To identify correlated evolution, we first analyzed our data using independent contrasts (Felsenstein 1985) based on the phylogeny of Higdon et al. (2007). Raw data were log-transformed before contrasts were calculated. We checked whether contrasts were correctly standardized by correlating them with their standard deviation (SD), which is the root of the sum of the involved branch lengths (Felsenstein 1985). Only contrasts in lactation length were significantly correlated with their SD ($r = 0.402, n = 34, P = 0.018$), so correlations reported for this variable need to be interpreted with caution (Revell 2010). Statistical significance was determined using parametric correlation coefficients.

To further identify the most likely evolutionary pathways connecting variables, we used two different approaches. First, we used the program DISCRETE (Pagel 1994) to conduct phylogenetic comparative analyses based on the phylogeny of Higdon et al. (2007), which contains all extant pinniped species, and the phylogeny of Arnason et al. (2006), which contains only 80% of all extant pinniped species excluding mostly eared seal species of the genus *Arctocephalus*. These two ultrametric phylogenies not only differ in the number of species included, but they also show some minor variations in branching patterns. We therefore analyzed both to test if our conclusions were robust to the choice of phylogeny. Unless stated otherwise, results presented are based on the complete phylogeny of Higdon et al. (2007). As a further procedure to check the robustness of our results, we partially

jack-knifed the phylogeny, removing single species or species pairs from this phylogeny to evaluate if any particular species exert a disproportionate influence on the results.

Because DISCRETE only allows the analysis of dichotomous variables, all continuous variables were dichotomized. For size dimorphism, all species with larger females were coded as 0 and those with larger males were coded as 1. When analyzing each sex separately, we categorized a male or female as large (1) if its body weight was above the sex-specific mean and as small (0) if the body weight was below the sex-specific mean. Following Caro et al. (2012), all species with sexual dimorphism in fur coloration were scored as 1 whereas all sexually monomorphic species were scored as 0. Other continuous variables were dichotomized using the median as a threshold, such that values below the median were scored as 0 and above as 1.

The maximum likelihood (ML) approach implemented in DISCRETE estimates the parameters of trait evolution by summing the likelihood over all possible states at each node of a phylogeny. Instead of estimating ancestral states, as implemented by maximum parsimony (MP) approaches, DISCRETE estimates the probabilities of each character state at each node. This more flexible approach is considered superior to MP (Pagel 1994) because it takes into account branch lengths and, by estimating rates of trait evolution along all branches, effectively controls for phylogenetic inertia. To find the most likely ancestral state, the likelihood of each state at the root of the tree was calculated using the “local option” (Pagel 1999). This approach allows to estimate the best simultaneous set of ancestral states on the tree. There are 2^n possible assignments of ancestral states of a binary character to n nodes. This option calculates the likelihood of each of them and identifies the single assignment of ancestral states to the n nodes that has the highest likelihood. To determine if the estimated likelihood of a given ancestral state was consistent, we ran the ancestral state reconstruction algorithm 20 times, allowing calculation of an average posterior probability and its associated standard error. We also used likelihood ratio (LR) tests, based on the χ^2 distribution with one degree of freedom (df), to evaluate if one ancestral state was significantly more likely than the other.

DISCRETE also uses LR tests to evaluate the null hypothesis (independent model, IM) that two traits evolved independently along the phylogeny against the alternative hypothesis that they show correlated evolution (dependent model, DM). Support for correlated evolution is found when the model of the two traits with correlated evolution fits the data significantly better than the null hypothesis. Given the limited number of extant pinniped species, several of the traits show relatively few changes along the phylogeny. We therefore conducted 100 simulations to determine the most appropriate number of degrees of freedom for the test of correlated evolution (Pagel 1994). These simulations suggested three degrees of freedom to be appropriate, and this was therefore

used throughout. DISCRETE also enables the analysis of evolutionary pathways between character states, outputting these in the form of flow diagrams. With two binary traits, eight transitions are possible. DISCRETE tests which of these are statistically significant by setting them, one by one, to zero. If a transition parameter is significant, setting it to zero will significantly reduce the likelihood of the DM. This establishes the most likely sequence of evolutionary changes and thereby allows tentative inferences about cause and effect, enhancing the explanatory power of comparative analyses (van Noordwijk 2002). Rather than restricting our analyses to those parameters predicted to change under different hypothesized pathways, we evaluated each parameter in turn to identify all of the eight possible evolutionary pathways and to establish the most likely one. The logic for establishing the most likely evolutionary sequence of events is that if trait “*a*” changed before trait “*b*” and trait “*b*” before trait “*c*,” then the most likely sequence is “*abc*” (Boerner and Krüger 2008).

Because the phylogenies contained only 35/28 extant species and branch lengths varied between 0.1 and 26 million years, we deployed the scaling parameter κ . This scaling parameter shortens longer branches relative to shorter ones, thus making it easier to find the global ML instead of getting trapped at local peaks of the likelihood surface (Pagel 1994). In our analyses, κ was estimated between 0.1 and 0.3, thus differences between branch lengths were reduced, resulting in a tree that was not strictly ultrametric but returned more consistent results in the ML search. To check whether key results were robust even without using κ , we repeated the analyses with $\kappa = 1$ and the results remained qualitatively the same.

As a second approach to find the most likely evolutionary pathways, we used phylogenetic confirmatory path analyses, a method recently described by von Hardenberg and Gonzalez-Voyer (2013). This approach uses directed acyclic graphs to specify models of evolutionary sequences and then tests the conditional probabilistic independences implied in the models while incorporating a phylogenetic hypothesis via the phylogenetic generalized least square method (Martins and Hansen 1997). This approach hence establishes a model of the best supported causal dependencies among a set of variables. Following the phylogenetic confirmatory path analysis method, we first defined 13 biologically relevant models of evolutionary sequences using data for our five continuous variables (Fig. 2), in contrast to the dichotomized data used for DISCRETE. Each model included a causal relationship of the variables SSD and harem size. For each model, we then derived a set of basic equations describing the probabilistic paths. That is, for each model, we first listed all sets of variables that have no direct connection (e.g., model A: (X1,X3), (X1,X4), (X1,X5), (X2,X4), (X2,X5), (X3,X5)). Subsequently, for each of these variables, we then listed the parent variables in the evolutionary sequence (e.g., model A: {X2}, {X3}, {X4}, {X1,X3}, {X1,X4}, {X2,X4}). Subsequently, we used these definitions to construct

a set of equations that describe the relationship between these independent variables, conditional on the parent variables (e.g., model A, first equation: $X3 \sim X2 + X1$; see Table S2 for all model descriptions and sets of probabilistic pathways). Using phylogenetic generalized least squares (function *gls* of R-package *nlme*), we corrected for the phylogenetic structure provided by the tree in Higdon et al. (2007). The significance level p_i of the variable (e.g., X1 in model A1) conditional of the parent variable in the model (e.g., X2 in model A2) was used for further analysis to compute Fisher’s *C*-statistic (von Hardenberg and Gonzalez-Voyer 2013). For each evolutionary sequence model, the *C*-statistic was computed and subsequently compared using the *C*-statistic Information Criterion (CICc) modified for small sample sizes. Thereafter, we used standard model selection procedure (akin to Akaike Information Criterion [AIC] model selection) to compare the different models of evolutionary pathways.

Results

INDEPENDENT CONTRAST ANALYSIS

We found strong evidence for multiple traits coevolving with both SSD and harem size, which we use as a proxy for the mating system. Contrasts in SSD were significantly correlated with every variable tested: harem size ($r = 0.661, n = 34, P < 0.001$), breeding latitude ($r = 0.429, n = 34, P = 0.011$), season length ($r = -0.543, n = 34, P < 0.001$), and lactation length ($r = -0.632, n = 34, P < 0.001$). Contrasts in harem size were significantly correlated with SSD (see above) and lactation length ($r = -0.516, n = 34, P = 0.002$) and were not related to breeding latitude ($r = 0.088, n = 34, P = 0.621$) and season length ($r = -0.297, n = 34, P = 0.088$). Analyzing male and female body weight separately, contrasts in male body weight were correlated with SSD ($r = 0.493, n = 34, P = 0.003$), harem size ($r = 0.456, n = 34, P = 0.007$), and breeding latitude ($r = 0.520, n = 34, P = 0.002$), but not with season length ($r = -0.221, n = 34, P = 0.209$) or lactation length ($r = -0.144, n = 34, P = 0.417$). Contrasts in female body weight showed no relationship with lactation length ($r = 0.296, n = 34, P = 0.089$) or with SSD ($r = -0.166, n = 34, P = 0.348$). There was also a correlation between male and female body weight contrasts ($r = 0.776, n = 34, P < 0.001$), reflecting the species-specific component of body size evolution. These results suggest that a whole suite of traits coevolved during the evolution of pinnipeds and that SSD was primarily driven by changes in male body size.

RECONSTRUCTION OF ANCESTRAL STATES

We found evidence for a suite of ancestral trait values (Table 1) pointing toward a polar origin of seals with both sexes being of the same size and the same pelage coloration, a promiscuous

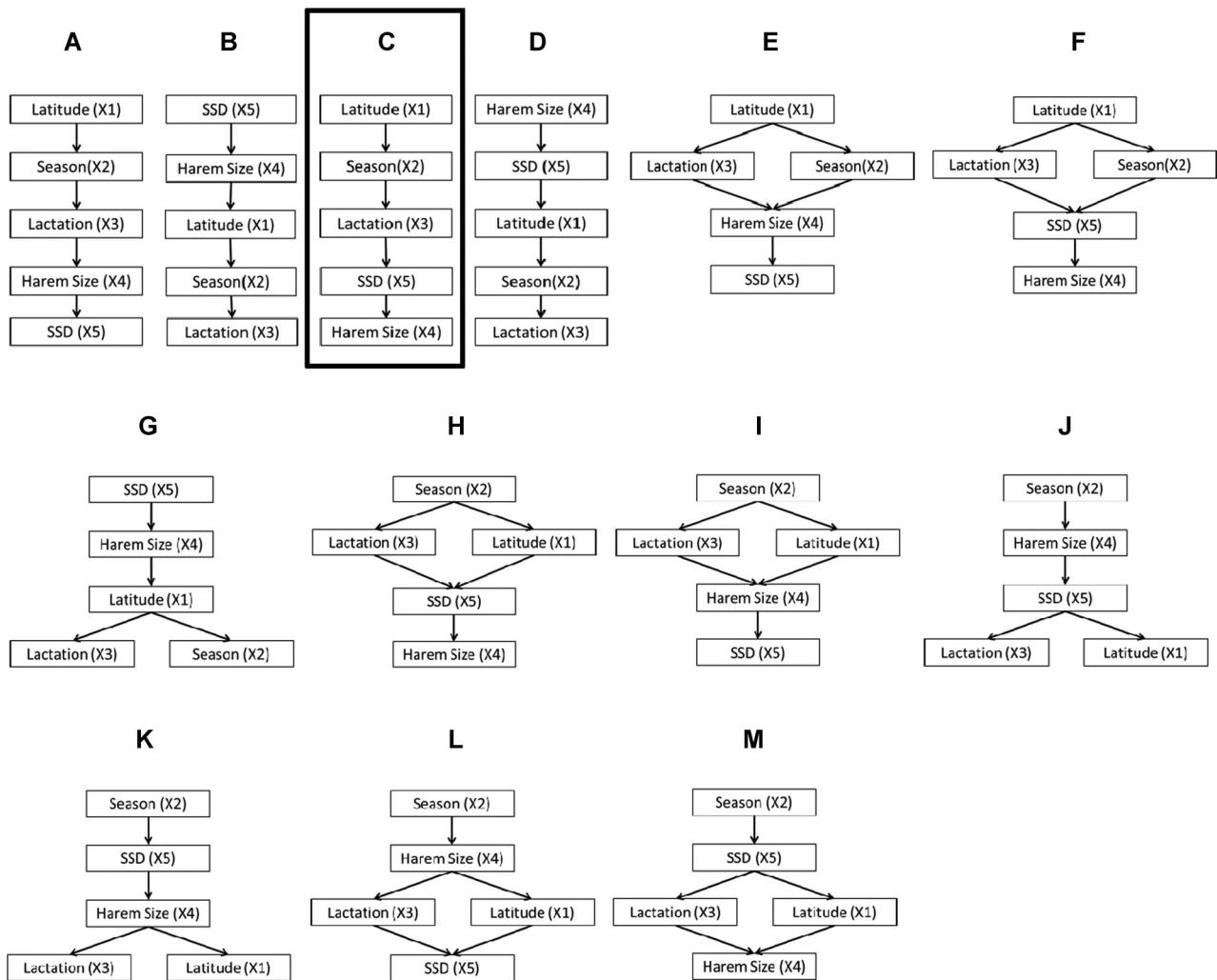


Figure 2. Set of candidate models for the phylogenetic confirmatory path analyses with the most likely model (C) being highlighted with a bold frame.

mating system with equal variance of reproductive success in both sexes, here called “promiscuity” as the ancestral breeding system with small female group sizes, aquatic copulation, breeding taking place on ice in the presence of terrestrial predation, shallow dives and short lactation periods and breeding seasons. Average probabilities of these ancestral states were in all cases significantly higher than 0.5, but LR tests were only significant for breeding on ice versus breeding on land ($\chi^2 = 3.965$, $df = 1$, $P = 0.046$), presence versus absence of terrestrial predation ($\chi^2 = 8.999$, $df = 1$, $P = 0.003$), and small versus large female groups ($\chi^2 = 4.265$, $df = 1$, $P = 0.039$). Shallow versus deep dives ($\chi^2 = 3.604$, $df = 1$, $P = 0.058$) and the presence/absence of pelage dimorphism ($\chi^2 = 2.869$, $df = 1$, $P = 0.090$) only showed trends.

EVOLUTIONARY PATHWAYS

We found evidence for coevolution between SSD and harem size, a proxy for the level of polygyny, using both the Higdon et al.

phylogeny ($IM = -25.673$, $DM = -20.693$, LR test = 9.960, $df = 3$, $P = 0.019$) and the Arnason et al. phylogeny ($IM = -23.211$, $DM = -18.682$, LR test = 9.058, $df = 3$, $P = 0.029$). However, it was not possible to directly distinguish between our alternative hypotheses. The most likely ancestral state (females \geq males and promiscuity) can be linked to the state displayed by most extant species (females $<$ males and polygyny) via either changes in mating system occurring first or changes in size dimorphism occurring first (Fig. 3, top panel). Moreover, rates of evolutionary change from the ancestral state to the alternative intermediate states were not significantly different from one another using both phylogenies (Higdon et al. $q_{12} = q_{13}$, LR test = 0.588, $df = 1$, $P = 0.443$; Arnason et al. $q_{12} = q_{13}$, LR test = 0.536, $df = 1$, $P = 0.464$), nor were there significant differences in the rates linking these intermediate states to the derived state (Higdon et al. $q_{24} = q_{34}$, LR test = 1.164, $df = 1$, $P = 0.281$; Arnason et al. $q_{24} = q_{34}$, LR test = 1.928, $df = 1$, $P = 0.165$). The correlation

Table 1. Most likely ancestral states for the variables included in the study.

Variable	Ancestral state	Average probability	SE	P	L-ratio	P
Sexual size dimorphism	Female \geq male	0.693	0.035	0.001	0.400	NS
Mating (promiscuity or polygyny)	Promiscuity	0.778	0.019	0.001	2.028	NS
Ice- or land-breeding	Ice-breeding	0.796	0.080	0.01	3.965	0.046
Terrestrial predator (yes or no)	Yes	0.743	0.062	0.001	8.949	0.003
Copulation (aquatic or land/ice)	Aquatic	0.818	0.024	0.001	1.671	NS
Diving depth (shallow or deep)	Shallow	0.762	0.049	0.001	3.604	0.058
Female group size (small or large)	Small	0.888	0.023	0.001	4.699	0.030
Latitude (temp/tropical or polar)	Polar	0.713	0.038	0.001	1.336	NS
Lactation period (short or long)	Short	0.575	0.017	0.001	0.362	NS
Breeding season (short or long)	Short	0.597	0.020	0.001	0.450	NS
Pelage dimorphism (yes or no)	No	0.930	0.006	0.001	2.869	0.090

Average probability refers to the posterior probability of a state = 0 or = 1 at the base of the phylogeny as calculated by DISCRETE under the local fossil likelihood command. The first column of P-values tests whether the average probability is significantly different from 0.5. The likelihood ratio (L-ratio) and its associated second column of P-values indicate whether an ancestral state is significantly more likely.

between male and female body weight was nonsignificant using evolutionary pathways (IM = -38.901, DM = -36.507, LR test = 4.788, df = 3, P = 0.188).

To disentangle the evolution of SSD and mating system, we next incorporated the remaining ecological and life-history variables into our analysis. Out of all variables tested, only the inclusion of breeding habitat allowed us to distinguish a most likely chain of evolutionary events, suggesting that SSD and mating system tightly coevolved. Figure 3 (middle panel) shows the predicted coevolutionary relationships between SSD and breeding habitat for the two phylogenies (Higdon et al. IM = -24.849, DM = -21.382, LR test = 6.934, df = 3, P = 0.074 and Arnason et al. IM = -24.251, DM = -20.157, LR test = 8.188, df = 3, P = 0.042). The most likely sequence from the ancestral state (females \geq males and breeding on ice) involves an initial change in SSD with males becoming bigger than females (Higdon et al. $q_{13} = 0$, LR test = 4.980, df = 1, P = 0.026 and Arnason et al. $q_{13} = 0$, LR test = 4.494, df = 1, P = 0.034) that is followed by a transition to breeding on land (Higdon et al. $q_{34} = 0$, LR test = 8.590, df = 1, P = 0.003 and Arnason et al. $q_{34} = 0$, LR test = 5.210, df = 1, P = 0.022).

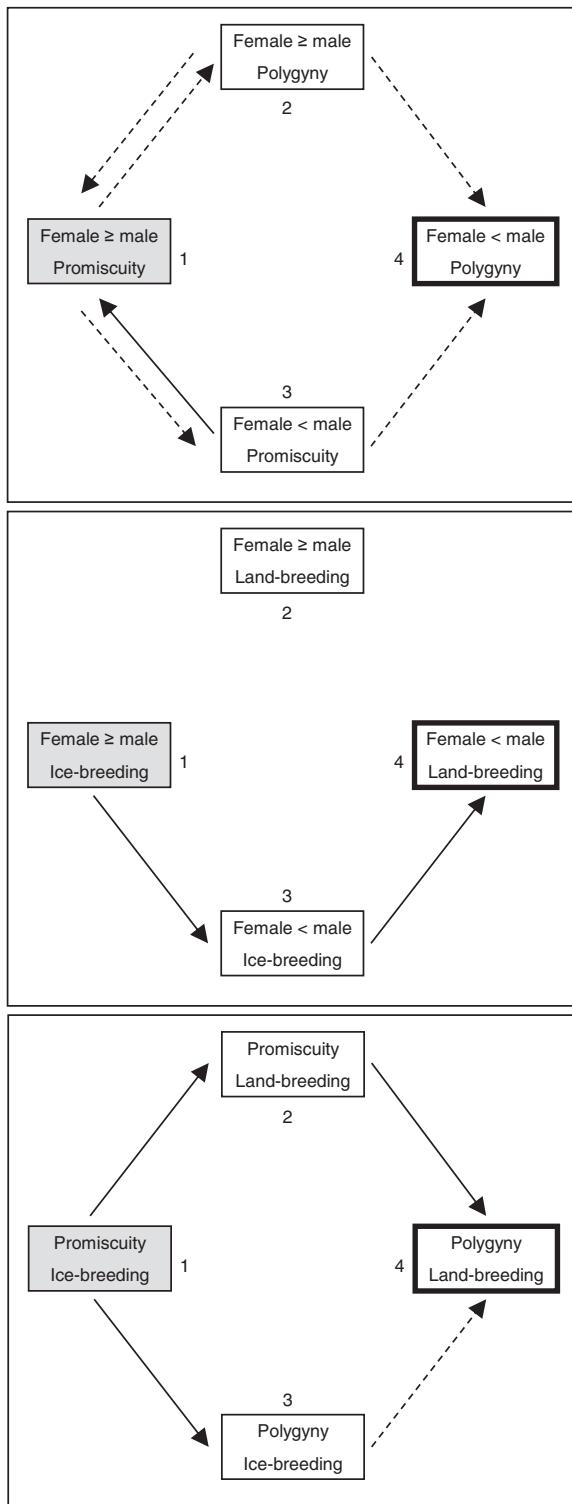
Figure 3 (bottom panel) shows the equivalent pathways for harem size and breeding habitat for both phylogenies (Higdon et al. IM = -28.303, DM = -22.799, LR test = 11.008, df = 3, P = 0.012 and Arnason et al. IM = -26.050, DM = -22.280, LR test = 7.540, df = 3, P = 0.057). From the ancestral state “promiscuity and breeding on ice,” significant pathways exist to both possible intermediate stages: “promiscuity and breeding on land,” Higdon et al. $q_{12} = 0$, LR test = 9.594, df = 1, P = 0.002 and Arnason et al. $q_{12} = 0$, LR test = 11.920, df = 1, P = 0.001 but also “polygyny and breeding on ice,” Higdon et al. $q_{13} = 0$, LR test = 6.168, df = 1, P = 0.013 and Arnason et al. $q_{13} = 0$, LR test = 7.404, df = 1, P = 0.007. However, from the intermediate

stage “promiscuity and breeding on land,” there was a significant pathway to the derived state under the Higdon et al. phylogeny ($q_{24} = 0$, LR test = 4.522, df = 1, P = 0.033), whereas there was only a weak trend from the other intermediate stage “polygyny and breeding on ice” to the derived state ($q_{34} = 0$, LR test = 2.888, df = 1, P = 0.089). Using the Arnason et al. phylogeny, the only potential pathway was from the intermediate stage “promiscuity and breeding on land” to the derived state ($q_{24} = 0$, LR test = 3.516, df = 1, P = 0.061). Hence, it is more likely that breeding habitat first changed and only as a consequence did harem size change under both phylogenies.

These results suggest that the most likely sequence of evolutionary events was that male-biased size dimorphism evolved first, followed by a transition to breeding on land and then the evolution of polygyny. This supports the hypothesis that SSD facilitated the evolution of polygyny and was not a consequence of it.

To evaluate the robustness of our analyses, we removed species one by one from the Higdon et al. phylogeny and tested if there was still evidence for correlated evolution between SSD and mating system and for the evolutionary pathways described above. Removing a single species, identical results were obtained regardless of the species in question. However, differences were obtained when both elephant seal species, *Mirounga angustirostris* and *M. leonina*, were removed together. In this case, we still found a statistical trend for SSD and mating system to coevolve (IM = -19.975, DM = -16.257, LR test = 7.436, df = 3, P = 0.059), but no evolutionary pathways could link the ancestral and the derived state. In addition, we found neither evidence of correlated evolution between SSD and breeding habitat (IM = -21.190, DM = -18.611, LR test = 5.158, df = 3, P = 0.161) nor between mating system and breeding habitat (IM = -21.207, DM = -19.536, LR test = 3.342, df = 3, P = 0.342).

Higdon et al. (2007) phylogeny



Arnason et al. (2006) phylogeny

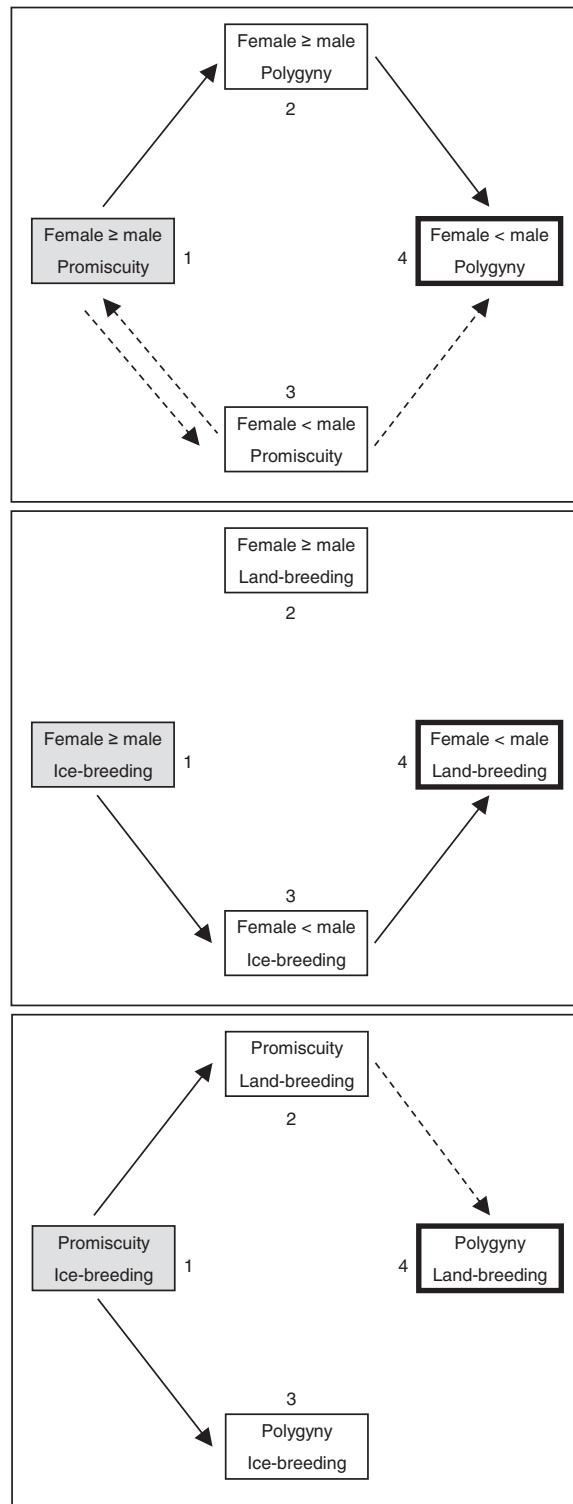


Figure 3. Evolutionary pathway diagrams depicting the most likely evolutionary pathways between sexual size dimorphism and mating system (top row), sexual size dimorphism and ice or land-breeding (middle row), and mating system and ice or land-breeding (bottom row). The three panels of the left were based on analyses using the phylogeny of Higdon et al. (2007), whereas the three panels on the right were based on analyses using the phylogeny of Arnason et al. (2006). Most likely ancestral states are shaded in gray and the box showing the extant state of the majority of pinniped species is highlighted in bold. Solid arrows indicate pathways that are significant at $P < 0.05$ or higher and dashed arrows show pathways approaching significance ($P < 0.1$). Numbers adjacent to boxes serve to facilitate the labeling of evolutionary pathways (q), that is, q_{12} means the pathway from box 1 to box 2.

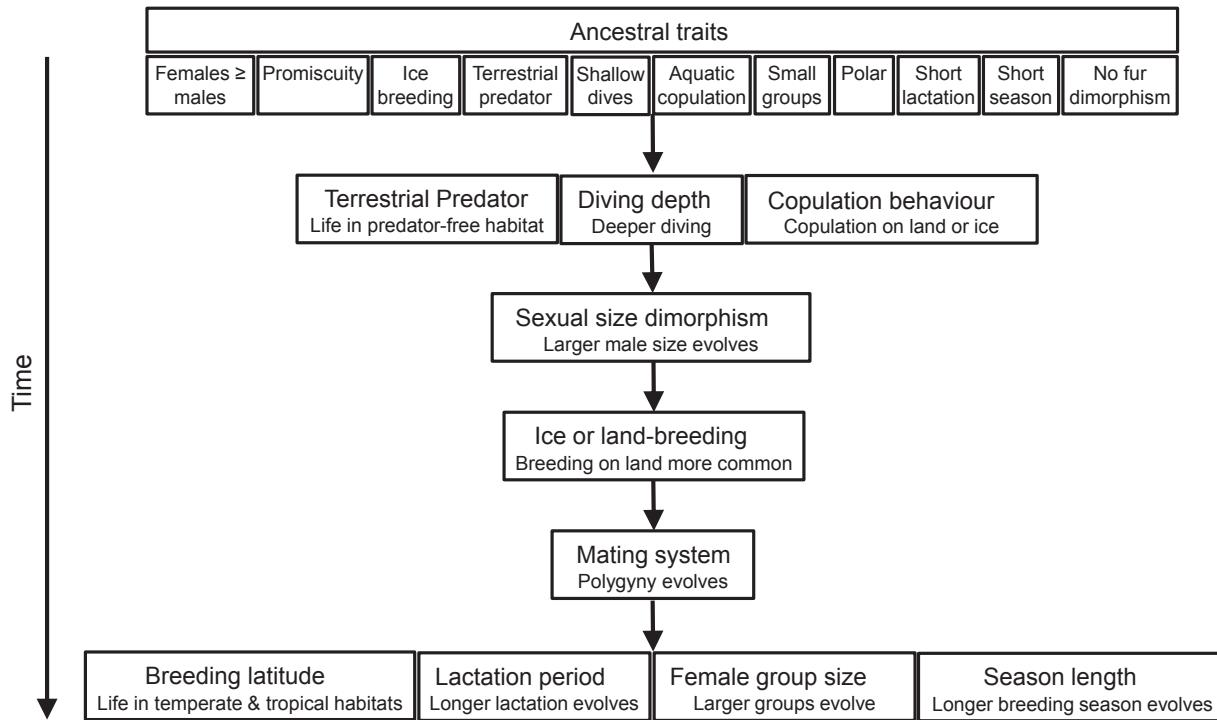


Figure 4. Most likely sequence of pinniped trait evolution. From the ancestral states shown at the top, we find evidence for initial changes in predator presence and copulation behavior, followed by the evolution of sexual size dimorphism. A subsequent shift toward breeding more on land then precedes the evolution of polygyny. The traits at the bottom are most likely consequences of these preceding evolutionary changes. We could not place the evolution of pelage dimorphism onto the evolutionary time line due to a lack of significant evolutionary pathways.

Using analogous arguments as for the investigation of the coevolutionary relationship between SSD and mating system, we constructed the most likely sequence of evolutionary changes during pinniped evolution for all traits under consideration (Fig. 4). The earliest of these events were that predator-free habitat was occupied, copulation moved from water to ice/land, and diving depths increased. These were followed by the transition from ancestrally female-biased size dimorphism to males becoming bigger than females. A subsequent shift toward breeding on land was then followed by the evolution of polygyny. The remaining ecological and life-history variables such as female group size, breeding latitude, length of the lactation period, and the length of the breeding season changed only after the evolution of both SSD and polygyny, making them probable downstream consequences rather than causes (Fig. 4).

PHYLOGENETIC CONFIRMATORY PATH ANALYSIS

We finally analyzed our data using a recently developed approach that makes full use of continuously distributed variables to allow the reconstruction of evolutionary events (von Hardenberg and Gonzalez-Voyer 2013). Out of the 13 models we considered, one model (Fig. 2, panel C) clearly had the highest support with a CICc weight of 0.816. No other model had a Δ CICc of less than

four and a model weight above 0.1 (Table 2). This model suggests that breeding latitude has a causal influence on breeding season length, which in turn causally influences lactation length having a causal influence on SSD. Crucially, SSD then is predicted to have a causal influence on harem size by the best model. Hence, the most likely evolutionary path is that SSD caused changes in harem size and not vice versa. In contrast to the evolutionary pathway analyses (Fig. 4), the traits breeding latitude, breeding season length, and lactation length were identified to induce change in SSD and harem size and not to change after the evolution of both SSD and harem size (see Fig. 2, panel C).

Discussion

Using two phylogenetically controlled comparative approaches, we attempted to tease apart the evolution of SSD and polygyny in a taxon that is frequently invoked to explain the power of sexual selection. We found the most likely evolutionary pathway to be one in which males first evolved to become larger than females, followed by the evolution of polygyny, regardless of which method was employed and which phylogenetic hypothesis was used. With due caution as to the moderate size of the taxon, our results support a scenario that stands in contrast to the traditional view of the

Table 2. Results of the model comparison of the phylogenetic confirmatory path analyses using CICc model selection.

Model	<i>C</i>	<i>q</i>	<i>k</i>	<i>P</i>	CICc	Δ CICc	CICc weight
C	12.32	9	6	0.420	37.518	0	0.816
D	17.06	9	6	0.147	42.263	4.745	0.076
A	18.27	9	6	0.108	43.466	5.948	0.042
F	14.33	10	6	0.280	43.492	5.974	0.041
G	20.09	9	7	0.127	45.293	7.775	0.017
E	18.99	10	6	0.089	48.156	10.639	0.004
I	18.99	10	6	0.089	48.156	10.639	0.004
J	34.68	9	7	0.002	59.877	22.359	0
K	40.63	9	7	<0.001	65.833	28.315	0
B	55.24	9	6	<0.001	80.440	42.922	0
L	58.04	10	6	<0.001	87.210	49.692	0
M	73.72	10	6	<0.001	102.887	65.369	0
H	83.51	10	6	<0.001	112.675	75.157	0

For a visualization of the different models, see Figure 2. The parameter *C* provides the value of the *C*-statistic, *q* gives the number of variables plus the number of paths in each model, *k* provides the number of independent tests in each model, and *P* provides the significance value testing for differences between the model and the real data (the less significant, the better the fit).

evolution of SSD (Fig. 1, see hypothesis 1 in the introduction plus Perez-Barberia et al. 2002 for a similar analysis in ungulates), but are in line with other examples where differences between the sexes are unlikely to be attributable only to sexual selection (Shine 1989; Mueller 1990; Irwin 1994; Radford and du Plessis 2003; Krüger 2005; Krüger et al. 2007). Our study further illustrates that a sequence of evolutionary events can be better understood within the overall context of evolutionary change in other ecological and life-history variables.

Independent contrast analyses revealed, in our experience, an unusually large number of significant correlations for a taxon of such a small size. This strongly suggests to us that a whole suite of traits coevolved during the evolution of pinnipeds. Interestingly, these correlations cannot be explained by simple allometric relationships between female size and variables such as breeding season length.

We have been able to deduce a most likely set of ancestral trait states for the Pinnpedia. These point toward a polar origin of seals with breeding on ice, a short lactation period and breeding season, aquatic copulation, shallow dives, small female groups, females as big or bigger than males, and promiscuity with equal variance in reproductive success in both sexes as the mating system. These ancestral states are reasonably consistent with expectations based on other studies (Deméré et al. 2003). For example, Pierotti and Pierotti (1980) make the case that in ice-breeding seals, females should be larger than males to avoid excessive heat loss. Moreover, there appears to be little polygyny in ice-breeding seals, probably due to the fact that males are less effective at monopolizing access to breeding females when mating occurs in the water (Bartholomew 1970). Similarly, the ancestral state of breeding in the presence of terrestrial predation

is consistent with contemporary levels of predation being high in ice-breeding seals (Caudron 1997). Both a longer breeding season and a longer lactation period might be seen as consequences of colonizing temperate and even tropical habitats later during pinniped evolutionary diversification. On current evidence, a nordic or even arctic origin of seals is also plausible (Deméré et al. 2003; Arnason et al. 2006; Rybczynski et al. 2009), given their close relationships to bears and possibly mustelids (Uhen 2007; Rybczynski et al. 2009; Nyakatura and Bininda-Emonds 2012).

Our evolutionary sequence analysis using DISCRETE placed three changes before changes in size dimorphism and mating system occurred: colonization of breeding habitat without terrestrial predators, a shift from aquatic copulation to copulation on ice/land, and the evolution of deeper diving depths. These three changes concern important traits that might have facilitated the evolution of male-biased SSD. Breeding on land without terrestrial predators would be expected to facilitate the clumping of females due to limited availability of suitable substrate in comparison to the vast foraging area available (Bartholomew 1970; Cassini 1999). A shift from aquatic copulation to mating on land could be especially important as males might force copulation onto females more easily on land than in the water, meaning that females might not be monopolized to the same degree when mating occurs aquatically (Harcourt et al. 2007). In addition, when receptive females congregate on land, it is easier for males to station among them than in the water. Aquatic mating makes it more difficult to herd females into a harem, but it does not preclude polygyny (Pörschmann et al. 2010; Meise et al. 2013) or associated behavior (Kunc and Wolf 2008). One possibility is that aquatic mating could initially have selected for larger male size

to dominate females (Clutton-Brock and Parker 1995). A shift toward deeper dives would select for larger size in both sexes as large body size allows for more oxygen storage relative to lean body mass and hence capacity for deeper diving (Weise et al. 2010). It would, however, also enable niche partitioning between the sexes if they were of different sizes as is observed in many vertebrate species (Ruckstuhl and Neuhaus 2005), including a number of seal species in which males dive deeper than females (Staniland 2005; Staniland and Robinson 2008; McIntyre et al. 2010). Changes in feeding ecology are an important driver of marine mammalian evolution (Uhen 2007) and hence it is not too far-fetched to suppose that this could be a trigger for the evolution of SSD. Once males were larger than females, and females bred in aggregations rather than being uniformly distributed, males could more effectively monopolize harems. This could also explain our finding that male and female body weight do not coevolve according to the evolutionary pathway analysis, which has been documented before (Lindenfors et al. 2002; Fitzpatrick et al. 2012a), but is in strong contrast to many other taxa (Abouheif and Fairbairn 1997; Lindenfors and Tullberg 1998).

The phylogenetic confirmatory path analyses based on the five continuous variables found that the most likely evolutionary path depicts breeding latitude, breeding season length, and lactation length to have had a causal influence on SSD which then led to changes in harem size. However, the key finding that SSD had a causal influence on harem size and not vice versa is the same as obtained with DISCRETE. Although the dichotomous variables were not included in this analysis, broad agreement between the two analyses leads us to tentatively propose that the polar origin of seals, whether expressed as ice versus land breeding or breeding latitude, may have initiated a whole suite of evolutionary changes in life history, ecology as well as SSD and harem size.

We do not see our results as contradictory to recent studies that have documented both higher rates of evolutionary change in traits under sexual selection (Fitzpatrick et al. 2012b) and strong selection on male body size in polygynous pinniped species (Lindenfors et al. 2002; Fitzpatrick et al. 2012a). It is abundantly clear that coevolution between mating system and SSD has been a powerful force in the evolution of the Pinnipedia. Nevertheless, our analyses suggest that the starting point might not have been the evolution of polygyny selecting for SSD, but instead the evolution of SSD may have occurred at an earlier time point, most likely due to natural and sexual selection acting on males. Interestingly, Poerschmann et al. (2010) reported that male body size was not a significant predictor of mating success in Galapagos sea lions despite this species being sexually size dimorphic, and Gonzalez-Suarez and Cassini (2014) recently found that variation in male reproductive success was not related to SSD across eight seal species, indicating that the assumption that male size has evolved

due to male–male competition alone might not be valid. Once males became bigger than females, however, this would have facilitated the evolution of polygyny in line with ecological factors promoting the clumping of females. Progressively, larger male body size and larger harems likely then coevolved in a positive feedback loop, consistent with the suggestion that both female grouping patterns and associated male harassment are decisive factors underlying the evolution of pinniped mating systems (Bartholomew 1970; Trillmich and Trillmich 1984; Riedman 1998; Cassini 2000).

Our evolutionary reconstruction with DISCRETE suggests that large female group sizes appear to have evolved relatively late in the inferred sequence of events. This is noteworthy given that several authors (Bartholomew 1970; Lindenfors et al. 2002; Fitzpatrick et al. 2012b) have previously assumed that large female aggregations are a prerequisite for the evolution of size dimorphism in pinnipeds. Although it appears that this may not necessarily be the case, female group size could still have played an important role at a later stage in reinforcing selection on large male body size (and hence SSD).

An important caveat to our study is that interpreting the results of comparative analyses necessarily involves some degree of speculation. Ultimately, comparative analyses are also dependent on the quality of the available comparative and phylogenetic data (Pagel 1999), although this is unlikely to be a problem for pinnipeds due to the scope and quality of data. Consequently, although we acknowledge inherent uncertainties in the interpretation of comparative analyses, we nevertheless feel that our results are worthwhile contemplating not only with regard to pinniped evolution but also more generally in relation to the forces that shape differences between the sexes. Our study also highlights the need for caution when assuming the direction of causality in the coevolution of two traits, even though that causality may initially seem intuitive.

ACKNOWLEDGMENTS

We are grateful to M. Boerner, P. Lindenfors, K. Reinhold, and two anonymous reviewers for helpful comments on the manuscript. This study was funded by a Heisenberg-Professorship to OK (KR 2089/2-1).

DATA ARCHIVING

The doi for our data is 10.5061/dryad.6p05C.

LITERATURE CITED

Abouheif, E., and D. J. Fairbairn. 1997. A comparative analysis of allometry for sexual size dimorphism: assessing Rensch's rule. *Am. Nat.* 149:540–562.

Andersson, M. B. 1994. *Sexual selection*. Princeton Univ. Press, Princeton, NJ.

Arnason, U., A. Gullberg, A. Janke, M. Kullberg, N. Lehman, E. A. Petrov, and R. Väinölä. 2006. Pinniped phylogeny and a new hypothesis for their origin and dispersal. *Mol. Phylogen. Evol.* 41:345–354.

Bartholomew, G. A. 1970. A model for the evolution of pinniped polygyny. *Evolution* 24:546–559.

Berta, A., and M. Churchill. 2012. Pinniped taxonomy: review of currently recognized species and subspecies, and evidence used for their description. *Mammal. Rev.* 42:207–234.

Boerner, M., and O. Krüger. 2008. Why do parasitic cuckoos have small brains? Insights from evolutionary sequence analyses. *Evolution* 62:3157–3169.

Caro, T., T. Stankowich, S. L. Mesnick, D. P. Costa, and K. Beeman. 2012. Pelage coloration in pinnipeds: functional considerations. *Behav. Ecol.* 23:765–774.

Cassini, M. H. 1999. The evolution of reproductive systems in pinnipeds. *Behav. Ecol.* 10:612–616.

—. 2000. A model of female breeding dispersion and the reproductive systems of pinnipeds. *Behav. Proc.* 51:93–99.

Caudron, A. K. 1997. Pinniped social systems: a review. *Mammalia* 61:153–160.

Clutton-Brock, T. H. 1988. *Reproductive success*. Chicago Univ. Press, Chicago.

—. 1991. The evolution of parental care. Princeton Univ. Press, Princeton.

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

Darwin, C. 1859. *The origin of species by means of natural selection*. John Murray, Lond.

—. 1871. *The descent of man, and selection in relation to sex*. John Murray, Lond.

Deméré, T. A., A. Berta, and P. J. Adam. 2003. Pinnipedimorph evolutionary biogeography. *Bull. Amer. Mus. Nat. Hist.* 279:32–76.

Fairbairn, D. J. 1997. Allometry for sexual size dimorphism: pattern and process in the coevolution of body size in males and females. *Ann. Rev. Ecol. Syst.* 28:659–687.

Fairbairn, D. J., W. U. Blanckenhorn, and T. Székely. 2007. Sex, size and gender roles: evolutionary studies of sexual size dimorphism. Oxford Univ. Press, Oxford, U.K.

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

Ferguson, S. H., and J. W. Higdon. 2006. How seals divide up the world: environment, life history, and conservation. *Oecologia* 150:318–329.

Fitzpatrick, J. L., M. Almbro, A. Gonzalez-Voyer, S. Hamada, C. Pennington, J. Scanlan, and N. Kolm. 2012a. Sexual selection uncouples the evolution of brain and body size in pinnipeds. *J. Evol. Biol.* 25:1321–1330.

Fitzpatrick, J. L., M. Almbro, A. Gonzalez-Voyer, N. Kolm, and L. W. Simmons. 2012b. Male contest competition and the coevolution of weaponry and testes in pinnipeds. *Evolution* 66:3595–3604.

Gonzalez-Suarez, M., and M. H. Cassini. 2014. Variance in male reproductive success and sexual size dimorphism in pinnipeds: testing an assumption of sexual selection theory. *Mammal. Rev.* 44: In press. doi: 10.1111/mam.12012.

Harcourt, R. G., J. J. Kingston, M. F. Cameron, J. R. Waas, and M. A. Hindell. 2007. Paternity analysis shows experience, not age, enhances mating success in an aquatically mating pinniped, the Weddell seal (*Leptonychotes weddelli*). *Behav. Ecol. Sociobiol.* 61:643–652.

Higdon, J. W., O. R. P. Bininda-Emonds, R. M. Beck, and S. H. Ferguson. 2007. Phylogeny and divergence of the pinnipeds (Carnivora: Mammalia) assessed using a multigene dataset. *BMC Evol. Biol.* 7:216.

Irwin, R. E. 1994. The evolution of plumage dichromatism in the new-world blackbirds: social selection on female brightness. *Am. Nat.* 144:890–907.

Krüger, O. 2005. The evolution of reversed size dimorphism in hawks, falcons and owls: a comparative analysis. *Evol. Ecol.* 19:467–486.

Krüger, O., and N. B. Davies. 2002. The evolution of cuckoo parasitism: a comparative analysis. *Proc. R. Soc. Lond. B* 269:375–381.

Krüger, O., N. B. Davies, and M. D. Sorenson. 2007. The evolution of sexual dimorphism in parasitic cuckoos: sexual selection or coevolution? *Proc. R. Soc. Lond. B* 274:1553–1560.

Krüger, O., M. D. Sorenson, and N. B. Davies. 2009. Does coevolution promote species richness in parasitic cuckoos? *Proc. R. Soc. Lond. B* 276:3871–3879.

Kunc, H. P., and J. B. W. Wolf. 2008. Seasonal changes of vocal rates and their relation to territorial status in male Galápagos sea lions (*Zalophus wollebaeki*). *Ethology* 114:381–388.

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

Lindenfors, P., B. S. Tullberg, and M. Biuw. 2002. Phylogenetic analyses of sexual selection and sexual size dimorphism in pinnipeds. *Behav. Ecol. Sociobiol.* 52:188–193.

Martins, E. P., and T. F. Hansen. 1997. Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* 149:646–667.

McIntyre, T., C. A. Tosh, J. Plötz, H. Bornemann, and M. N. Bester. 2010. Segregation in a sexually dimorphic mammal: a mixed-effects modelling analysis of diving behaviour in southern elephant seals. *Mar. Ecol. Prog. Ser.* 412:293–304.

Meise, K., O. Krüger, P. Piedrahita, and F. Trillmich. 2013. Site fidelity of male Galápagos sea lions: a lifetime perspective. *Behav. Ecol. Sociobiol.* 67:1001–1011.

Mueller, H. C. 1990. The evolution of reversed sexual dimorphism in size in monogamous species of birds. *Biol. Rev.* 65:553–585.

Nyakatura, K., and O. R. P. Bininda-Emonds. 2012. Updating the evolutionary history of Carnivora (Mammalia): a new species-level supertree complete with divergence time estimates. *BMC Biol.* 10:12.

Pagel, M. D. 1994. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc. R. Soc. Lond. B* 255:37–45.

—. 1999. Inferring the historical patterns of biological evolution. *Nature* 401:877–884.

Perez-Barberia, F. J., I. J. Gordon, and M. D. Pagel. 2002. The origins of sexual dimorphism in body size in ungulates. *Evolution* 56:1276–1285.

Pierotti, R., and D. Pierotti. 1980. Effects of cold climate on the evolution of pinniped breeding systems. *Evolution* 34:494–507.

Pörschmann, U., F. Trillmich, B. Mueller, and J. B. W. Wolf. 2010. Male reproductive success and its behavioural correlates in a polygynous mammal, the Galapagos sea lion (*Zalophus wollebaeki*). *Mol. Ecol.* 19:2574–2586.

Radford, A. N., and M. A. du Plessis. 2003. Bill dimorphism and foraging niche partitioning in the green woodhoopoe. *J. Anim. Ecol.* 72: 258–269.

Revell, L. J. 2010. Phylogenetic signal and linear regression on species data. *Meth. Ecol. Evol.* 1:319–329.

Riedman, M. 1998. *The pinnipeds: seals, sea lions, and walruses*. Univ. of California Press, Berkeley, CA.

Ruckstuhl, K. E., and T. H. Clutton-Brock. 2005. Sexual segregation and the ecology of the two sexes. Pp. 3–8 in K. E. Ruckstuhl and P. Neuhaus, eds. *Sexual segregation in vertebrates: ecology of the two sexes*. Cambridge Univ. Press, Cambridge.

Ruckstuhl, K. E., and P. Neuhaus. 2005. *Sexual segregation in vertebrates: ecology of the two sexes*. Cambridge Univ. Press, Cambridge.

Rybczynski, N., M. R. Dawson, and R. H. Tedford. 2009. A semi-aquatic Arctic mammalian carnivore from the Miocene epoch and origin of Pinnipedia. *Nature* 458:1021–1024.

Shine, R. 1989. Ecological causes for the evolution of sexual dimorphism: a review of the evidence. *Q. Rev. Biol.* 64:419–461.

Staniland, I. J. 2005. Sexual segregation in seals. Pp. 53–71 in K. E. Ruckstuhl and P. Neuhaus, eds. *Sexual segregation in vertebrates: ecology of the two sexes*. Cambridge Univ. Press, Cambridge.

Staniland, I. J., and S. L. Robinson. 2008. Segregation between the sexes: antarctic fur seals, *Arctocephalus gazella*, foraging at South Georgia. *Anim. Behav.* 75:1581–1590.

Székely, T., J. D. Reynolds, and J. Figuerola. 2000. Sexual size dimorphism in shorebirds, gulls, and alcids: the influence of sexual and natural selection. *Evolution* 54:1404–1413.

Thomas, G. H., R. P. Freckleton, and T. Székely. 2006. Comparative analyses of the influence of developmental mode on phenotypic diversification rates in shorebirds. *Proc. R. Soc. Lond. B* 273:1619–1624.

Trillmich, F. 1996. Parental investment in pinnipeds. Pp. 533–577 in J. S. Rosenblatt and C. T. Snowdon, eds. *Parental care: evolution, mechanisms, and adaptive significance*. Academic Press, San Diego, CA.

Trillmich, F., and K. G. K. Trillmich. 1984. The mating systems of pinnipeds and marine iguanas—convergent evolution of polygyny. *Biol. J. Linn. Soc.* 21:209–216.

Trivers, R. L. 1972. Parental investment and sexual selection. Pp. 136–179 in B. Campbell, ed. *Sexual selection and the descent of man*. Aldine, Chicago.

Uhen, M. D. 2007. Evolution of marine mammals: back to the sea after 300 million years. *Anat. Rec.* 290:514–522.

van Noordwijk, A. J. 2002. The tale of the parasitic cuckoos. *Nature* 416:687–690.

von Hardenberg, A., and A. Gonzalez-Voyer. 2013. Disentangling evolutionary cause-effect relationships with phylogenetic confirmatory path analysis. *Evolution* 67:378–387.

Weise, M. J., J. T. Harvey, and D. P. Costa. 2010. The role of body size in individual-based foraging strategies of a top marine predator. *Ecology* 91:1004–1015.

Associate Editor: P. Lindenfors

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Raw data used in this study.

Table S2. Model definition and sets of basic equations for the phylogenetic confirmatory path analysis.