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Macroevolution of sexually selected weapons: weapon evolution in chameleons

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Abstract

The evolution of sexually selected traits is a major topic in evolutionary biology. However, large-scale evolutionary patterns in these traits remain understudied, especially those traits used in male–male competition (weapons *sensu lato*). Here, we analyze weapon evolution in chamaeleonid lizards, both within and between the sexes. Chameleons are an outstanding model system because of their morphological diversity (including 11 weapon types among ~220 species) and a large-scale time-calibrated phylogeny. We analyze these 11 traits among 165 species using phylogenetic methods, addressing many questions for the first time in any group. We find that all 11 weapons have each evolved multiple times and that weapon origins are generally more frequent than their losses. We find that almost all weapons have each persisted for >30 million years (and some for >65 million years). Across chameleon phylogeny, we identify both hotspots for weapon evolution (up to 10 types present per species) and coldspots (all weapons absent, many through loss). These hotspots are significantly associated with larger male body size, but are only weakly related to sexual-size dimorphism. We also find that weapon evolution is strongly correlated between males and females. Overall, these results provide a baseline for understanding large-scale patterns of weapon evolution within clades.

Keywords: lizard, macroevolution, male–male competition, phylogeny, sexual dimorphism, sexual selection

Sexually selected traits include many spectacular features of animals. These include beautiful songs, massive weapons, and dazzling colors (Andersson, 1994). More broadly, sexual selection seems to be an important driver of phenotypic diversity in many animal clades (Andersson, 1994; Darwin, 1871). Because of this, sexual selection is a major research topic in evolutionary biology.

There are two important but relatively understudied topics in the study of sexual selection. First, the evolution of weapons used in male–male competition remains understudied in recent sexual selection research (relative to the study of female choice), as shown in a quantitative analysis of the literature (McCullough et al., 2016). In many ways, this research emphasis on female choice is well justified. For example, the question of why females prefer males with exaggerated traits is puzzling, especially when these traits may seem to offer little obvious benefit to the female (e.g., Jones & Ratterman, 2009; Kirkpatrick & Ryan, 1991; Kokko et al., 2003). At the same time, a recent survey suggested that nearly half of all surveyed sexually selected traits may have arisen through male–male competition, and not female choice (Wiens &Tuschhoff, 2020), or at least in chordates (Tuschhoff &Wiens, 2023). Therefore, focusing exclusively on female choice is not sufficient to understand all sexually selected traits.

The second understudied topic is the evolution and diversity of sexually selected traits at relatively large phylogenetic scales (review in Wiens & Tuschhoff, 2020). Analyses within populations and closely related species are an appropriate

scale to study the process of sexual selection itself. However, understanding larger-scale patterns of trait diversity and evolution requires a broader focus. For example, it might be that the sexually selected traits seen among living species have evolved only recently. Alternatively, they may tend to evolve tens of millions of years ago and persist to the present day. This is difficult to determine without conducting larger-scale analyses that allow these deeper patterns to be revealed.

Given that these two topics are understudied, their intersection is especially poorly known, specifically large-scale evolutionary patterns in sexually selected weapons. Emlen (2008) and Rico-Guevara and Hurme (2019) have provided excellent reviews of weapon evolution in animals. Emlen (2008) highlighted the impressive diversity in weapon morphology among closely related species, but the mechanisms underlying this diversity remain poorly studied (McCullough et al., 2016). Note that here and throughout, we use "weapons" in the broad sense to refer to traits used in male–male competition (Emlen, 2008; McCullough et al., 2016). These can include traits used directly in physical combat, traits used only to intimidate rivals (i.e., signals), or traits used to do both.

We suggest that the following questions remain relatively unexplored for sexually selected traits in general, but especially for weapons. Many of these questions (1–4) were discussed by Wiens and Tuschhoff (2020). (1) For a given trait, how many times does it evolve, and what is the balance between gains and losses? For example, does it originate

once and is it then lost repeatedly among some species? Or does it evolve multiple times without being lost? Are there any factors that can explain the observed balance between gains and losses? (2) How long do these traits last? Are these weapons relatively unstable and evolutionarily short lived, or can they evolve tens of millions of years ago and persist to the present day? (3) In a given group of animals, there can be a single weapon or several (Emlen, 2008). Are there "hotspots" for weapon evolution in a given group, specifically, clades in which multiple weapons have evolved or accumulated in the same species? Alternatively, are there coldspots, in which most or all weapons are lacking? What other variables might explain these hotspots and coldspots? For example, in lizards, many sexually selected characters that play an important role in intrasexual territorial displays (i.e., dichromatism, head width, dorsal and tail crests/spines, rostral appendages) are associated with other variables, specifically large body size and male-biased sexual size dimorphism (SSD) (Juri et al., 2018; Stuart-Fox & Ord, 2004). (4) Similarly, do multiple weapons tend to evolve in an "arms race" among conspecific males (e.g., Emlen et al., 2005; Parker, 1983)? That is, do different weapons tend to be positively correlated in their distribution among species? Such a pattern could help explain the presence of both hotspots and coldspots for weapon evolution within a group. Alternatively, are there trade-offs among weapons, such that clades tend to have one weapon type but not another? These trade-offs might arise if there are costs to maintaining or developing multiple weapons, especially ones that are physically adjacent. For example, Emlen (2001) documented trade-offs among dung beetle species in the size of horns in different locations. Yet, Emlen et al. (2005) suggested that there was an overall pattern of escalation in weapon number among 48 species of dung beetles, resulting in species with up to five different horn types evolving from an ancestor with only one. (5) Another major aspect of weapon macroevolution is their correlated and/or decoupled evolution between the sexes. Weapons are used in male–male competition over females, but are often present in females also (e.g., Kraaijeveld 2014; Kraaijeveld et al., 2007; Robinson & Kruuk, 2007; Stankowich & Caro, 2009; Tobias et al., 2012). Is the presence of weapons in females associated with their presence in males? Do weapons evolve in both sexes at the same time, possibly followed by losses in females (given genetic correlations between males and females; e.g., Lande, 1980; Lande & Arnold, 1985), as might be expected for weapons used primarily for male-male combat? Alternatively, do females gain weapons after males, which may suggest some use of the weapon by females for competition for resources or mates (e.g., Clutton-Brock 2007, 2009; Cornwallis & Birkhead, 2007)? Does the number of gains and losses differ between males and females? What about the persistence of these weapons over time? There have been some macroevolutionary studies comparing the evolution of sexually selected traits between males and females (e.g., Burns, 1998; Ord and Stuart-Fox, 2006; Wiens, 1999). However, most of the questions listed above have not been addressed, and especially not for weapons.

Nevertheless, there have been several macroevolutionary studies of weapons, even if they did not address the five questions above. We briefly describe these below. Emlen et al. (2005) examined the evolution of five horn types in dung beetles (see Discussion) and found repeated gains of most weapon types but relatively few losses, in both males and females, except for one horn type that evolved once and was lost repeatedly. Rico-Guevara and Hurme (2019) provided an outstanding review of weapon evolution across animals and suggested several intriguing generalities about weapons used for within-species combat (such as their often evolving as paired elaborations of existing skeletal structures near the head in males of terrestrial taxa). These ideas could be tested with future statistical phylogenetic analyses. Similarly, Arbour and Zanno (2018) found that the evolution of weapons on the tail in amniotes (but not necessarily sexually selected ones) is correlated with large body size, herbivory, and body armor. Previous studies have also examined the correlates of the evolution of sexually selected weapons in artiodactyls. Caro et al. (2003) found that different horn morphologies were associated with different social systems and fighting strategies in bovids and cervids (e.g., straight horns in monogamous, solitary species). Bro-Jørgensen (2007) found that male horn length in bovids was positively related to breeding-group size, a measure of the potential for polygyny. An analysis of weapon evolution in harvestmen (Arthropoda: Opiliones) found a correlation between sexual dimorphism and male dimorphism (i.e., variation in weapon size among males; Buzatto et al., 2014). Emberts et al. (2021) showed that weapon performance (i.e., damage inflicted) predicted the evolution of weapon morphology among species in coreid bugs. An analysis of three insect clades (Emberts & Wiens, 2021) found that sexually selected weapons do not appear to accelerate diversification. Menezes and Palaoro (2022) inferred that flight impedes the evolution of weapons (bony spurs) in birds. This list of studies is not a systematic or comprehensive survey. Instead, our point is that there have been macroevolutionary studies on various diverse questions related to sexually selected weapons (or weapons in general), but few addressing the five questions outlined above.

Chamaeleonid lizards (chameleons hereafter) offer an exciting model system to address macroevolutionary questions about weapon evolution. First, chameleons have a diversity of morphological structures that are often sexually dimorphic (Figure 1), including horns, crests, casques, and spines (Table 1). Behavioral studies show that many of these structures function in male combat (or male-male competition in general), at least in some well-studied species (Table 1). Second, females of many species have structures similar to their male counterparts (Anderson & Higham, 2014). Thus, they offer a system in which to test for relationships between male and female weapon evolution. Third, despite having many species (219 described species; Uetz et al., 2021), their phylogeny is well known. For example, a time-calibrated tree based on nuclear and mitochondrial genes is available, a tree that includes ~79% of described chamaeleonid species (Tolley et al., 2013). This tree makes it possible to do large-scale phylogenetic analyses with extensive species-level sampling.

Methods

Data collection

We obtained morphological data (weapon presence/absence) on both males and females of each species from two main sources (Glaw & Vences, 2006; Tilbury, 2010). These books contain extensive and detailed descriptions of 199 of the 219 currently described chamaeleonid species (Uetz et al., 2021). However, descriptions of both males and females were available for only 165 species. One source included information
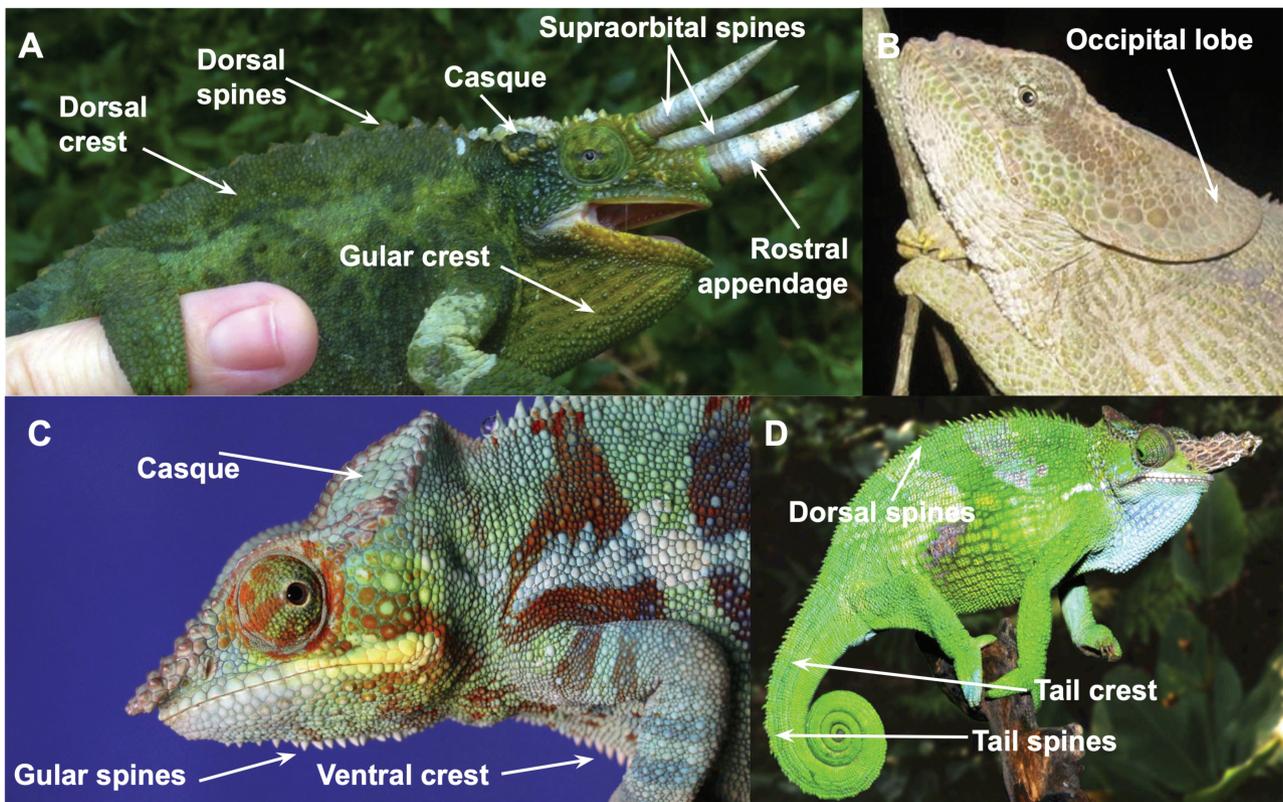
Figure 1. Weapon characters investigated in this study. See Table 1 for description of each character and information on their putative functions. Example species that exhibit one or more of these characters include: (A) *Trioceros jacksonii*, (B) *Calumma brevicorne*, (C) *Furcifer pardalis*, and (D) *Kinyongia multituberculata*. Photograph (A) is by Brenden Holland, (B) by John J. Wiens, (C) by Pierre Bamin, and (D) by Raimun Schlager. All photographs are used with permission.

on species from Madagascar (Glaw & Vences, 2006), whereas the other had information on species in other regions (especially Africa; Tilbury, 2010). We used the detailed species descriptions from these sources to score 11 weapon characters for both males and females (Figure 1) and confirmed these descriptions with illustrations when possible. These characters are summarized in Table 1, along with existing information on the function of these features. Each character was coded as present (1) or absent (0) in each sex in each species. These characters were selected given their use in intraspecific aggressive exchanges between males that are competing for females, at least in well-studied species (e.g., Karsten et al., 2009; Van Kleeck et al., 2018). Two characters included in this list have no known function in intraspecific behavior (tail crests and tail spines). However, these may be extensions of dorsal crests and spines, and so might share the same function. We know of no relevant behavioral studies on species with these two characters.

Note that there is evidence that most of these traits are used in male–male competition (Table 1), but some may also be used in female choice. In some cases, the same trait may be used in male–male competition in some species but in female choice in others (e.g., Karsten et al., 2009). In short, we have evidence that these traits are used in male–male competition in at least some taxa, but additional uses are also possible (which do not rule out their use as weapons).

We also used these sources (Glaw & Vences, 2006; Tilbury, 2010) to obtain data on the maximum adult snout–vent length (SVL) for each sex for each species. SVL is a standard measure of body size in lizards. We obtained SVL data for 148

species for males and 137 for females; 133 species had data for both sexes. We then calculated an sexual size dimorphism ratio for these 133 species. We used the formula from Lovich and Gibbons (1992):

$$SSD = ((L/S) - 1)$$

where L is the maximum body size for the larger sex, and S is the maximum body size for the smaller sex. The ratio is then multiplied by 1 if the female is the larger sex and –1 if the male is larger. Therefore, the ratio will be negative for species with male-biased SSD (males larger) and positive for species with female-biased SSD (females larger).

Statistical analyses

For all phylogenetic analyses, we used the time-calibrated phylogeny from Tolley et al. (2013: Figure 1). This tree included 174 described chamaeleonid species, including all 165 species for which we obtained weapon data for both males and females. The tree was based on a concatenated analysis of multiple nuclear and mitochondrial markers and was strongly supported across almost all nodes. This latter pattern suggests that analyzing a set of alternative trees from that study would yield similar results to our main findings here. Therefore, we did not perform an analysis across a distribution of trees (and no such distribution was available). We trimmed this tree prior to analysis by removing species for which weapon data were not available using PAUP* 4.0b10 (Swofford, 2002). We performed all comparative analyses using R version 3.2.2 (R Core Team, 2020). The trait data analyzed are in Supplementary Dataset S1, and the phylogeny

Table 1. Summary of characters investigated in this study, including descriptions and inferred function related to male–male competition. In many chameleon species, these characters show sexual dimorphism in either presence/absence or size (i.e., females lack the structure or have a smaller version of the structure; Glaw & Vences, 2006; Tilbury, 2010). Characters are illustrated in Figure 1. "Male–male combat" refers to the direct physical interactions that occur between males during contests for territory and mates. "Aggressive display" refers to the behaviors displayed by males during male-male contests, before direct physical contact occurs. ªVariation in the trait can help explain which males win contests (e.g., Karsten et al., 2009; Stuart-Fox et al., 2006), but it is unclear if the trait functions primarily in combat or primarily as part of the display. Tail crests and tail spines are sexually dimorphic characters, but we are not aware of any studies that have investigated their function.

Character	Description	Function	References
Casque	Part of the head that projects backward behind the eyes	Aggressive display[a]	Stuart-Fox et al. (2006); Karsten et al. (2009)Measey et al. (2009)Da Silva et al. (2014)
Rostral appendage	Granular scales or annulated horns protruding from rostrum	Male–male combat[a]	Rand (1961); Karsten et al. (2009)Van Kleeck (2016)
Supraorbital spines	Spines, cones, or horns located above eye	Male–male combat	Rand (1961); Van Kleeck (2016)
Occipital lobe	Ear-like flaps along the lateral sides of the casque	Aggressive display	Glaw and Vences (2006)
Dorsal crest	Raised keel along vertebral column	Aggressive display	Raxworthy (1991)
Dorsal spines	Cones or tubercles along dorsal crest	Aggressive display	Raxworthy (1991); Karsten et al. (2009)
Gular crest	Pronounced pouch beneath mandible	Aggressive display	Ligon (2014); Van Kleeck et al. (2018)
Gular spines	Cones or tubercles along gular crest	Aggressive display	Ligon (2014); Van Kleeck et al. (2018)
Ventral spines	Midline crest of tubercles along midline of belly	Aggressive display	Stuart-Fox et al. (2006)
Tail crest	Raised keel just anterior to tip of the tail	Unknown	NA
Tail spines	Cones or tubercles that extend along the top of the tail	Unknown	NA

is in Supplementary Dataset S2. The R code used is provided in Supplementary Dataset S3.

Gains and losses

To estimate the number of gains and losses for each trait, we used two methods. First, we reconstructed the evolution of each character using maximum likelihood with the "ace" function in the R package *ape* version 5.5 (Paradis et al., 2004). We also mapped characters onto the phylogeny using stochastic character simulations along each branch with the "make.simmap" function in the R package *phytools* (Revell, 2012). To produce the stochastic character maps, we simulated 100 maps and generated a summary map for each character. We then repeated our statistical analyses that involved ancestral reconstructions using the first 10 simulated character maps, to evaluate the sensitivity of these results.

Prior to conducting reconstructions, we found the best-fitting model of evolution for each character. We compared the fit of the equal-rates model (ER) and all-rates-different model (ARD). Under the ER model, the transition rates for gains (0–1) and losses (1–0) are equal. For the ARD model, gains and losses have different rates. We compared the Akaike information criterion (AIC; Akaike, 1974) for each model and considered the model with the lowest AIC as the best fitting. The ARD model typically had the best fit (Supplementary Tables S1–S2).

Nevertheless, inspection of ancestral reconstructions from both models revealed some problematic reconstructions for some characters for the ARD model. For all characters, we performed ancestral-state reconstructions under both the ER model (Figures 2–4, Supplementary Figures S1–S8) and ARD (Supplementary Figures S9–S19). Reconstructions of states that were relatively widespread among species (e.g., casques, rostral appendages, and dorsal crests) were similar under both ER and ARD models. However, for character states that were relatively rare among species (e.g., supraorbital spines, ventral

spines, and gular spines), the ARD model reconstructed these states as present at the root of the tree, with multiple subsequent losses then required to explain their absence in most species. This type of pattern has long been recognized as an erroneous inference associated with applying ARD models to characters with rare states (Schluter et al., 1997). Therefore, we chose to use the ER model for further analyses. Note that use of an ER model does not guarantee that there will be a similar number of gains and losses reconstructed for each character, as our results demonstrate.

Using both the ancestral-state reconstructions and stochastic character maps, we estimated the number of gains and losses for each trait for each sex. We assigned to each node the state with the highest proportional likelihood. A gain was inferred on a branch when a character state (e.g., casque) that was estimated to be absent at the beginning of the branch was inferred to be present by the end of that branch (i.e., comparing the node at the beginning of the branch to the node at the end of the branch). A loss was inferred when a trait that was initially present was inferred to be absent by the end of the branch. We then tallied the inferred number of gains and losses for each character across the tree. Gains included the initial origin of each trait (first-time gains) or secondary gains (characters regained after a loss). We tested for differences in numbers of gains and losses between males and females, and also differences between numbers of gains and losses for each sex using Welch's nonparametric two-sample *t*-tests (in R).

Ages of traits

We estimated the age of the initial origin of each character (the age of the oldest node on which the trait was inferred to be present), to determine how long these traits can persist over time. We also calculated the mean and the maximum age of the gains of each character. We then performed linear regression among traits to determine if the mean and
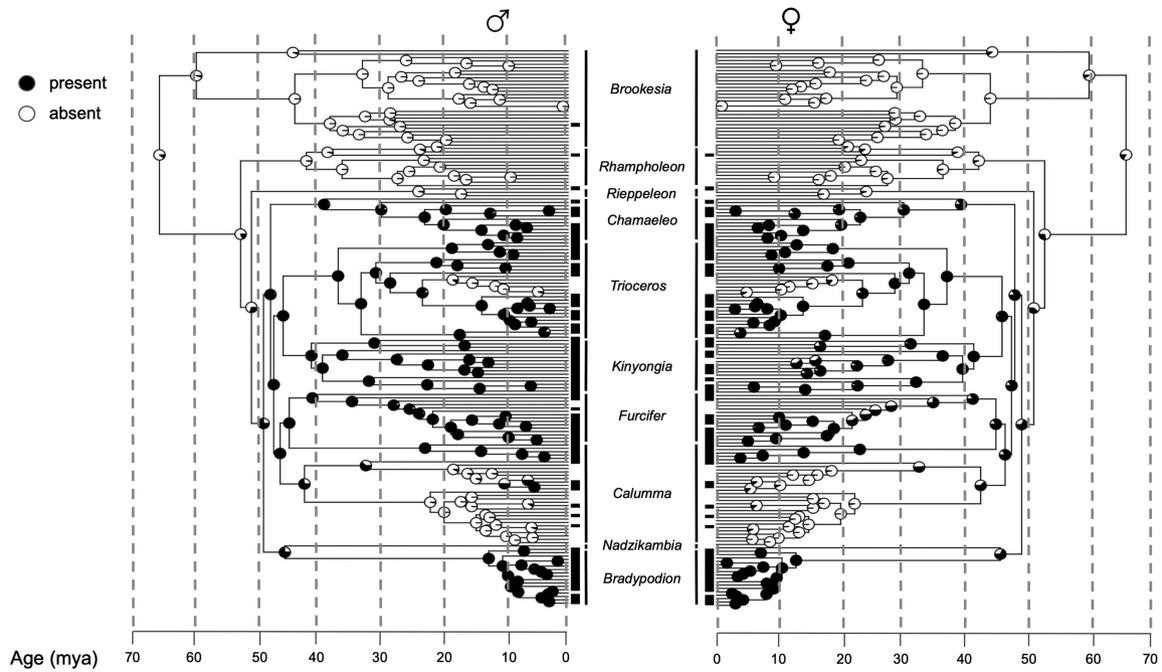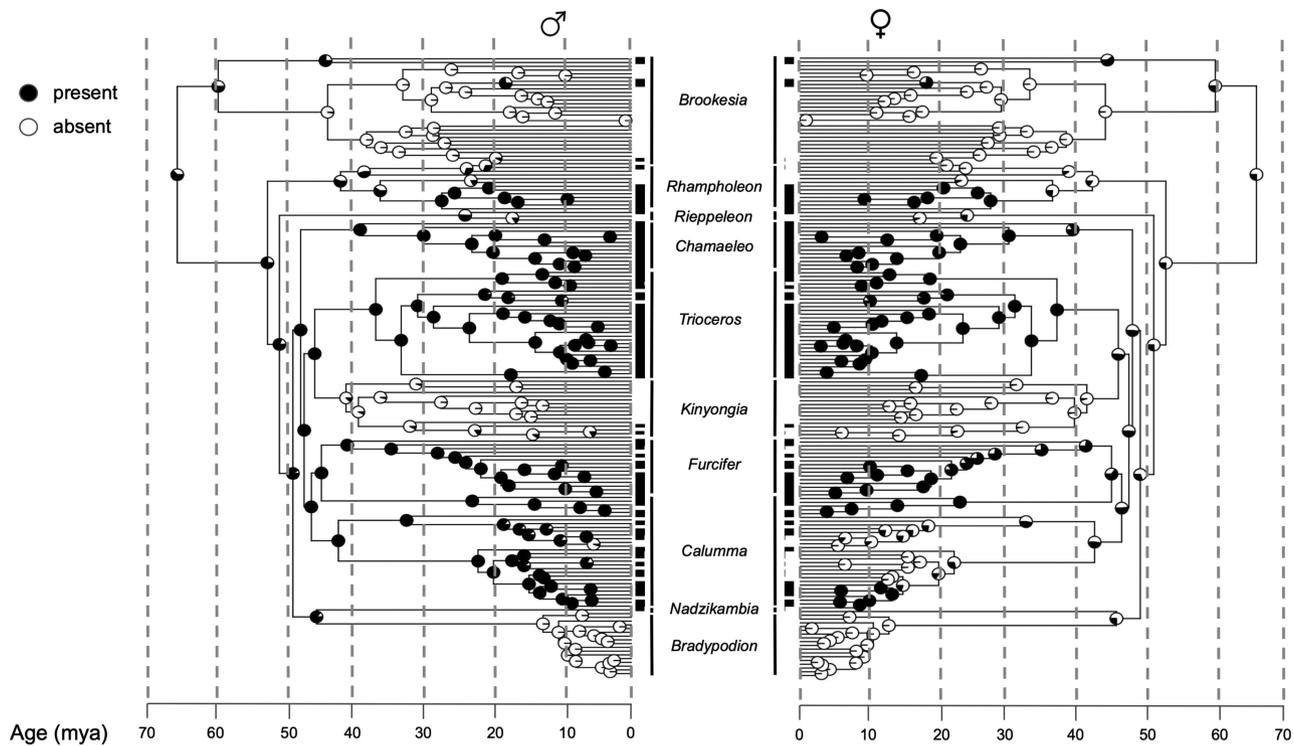
Figure 2. Evolution of casques in males (left) and females (right). Results shown are for the "equal rates" (ER) model; results for the all-rates-different (ARD) model are shown in Supplementary Figure S9. Proportional likelihoods of each state are shown in pie diagrams at each node (black = presence; white = absence). The black rectangles indicate that the casques are present in extant species.
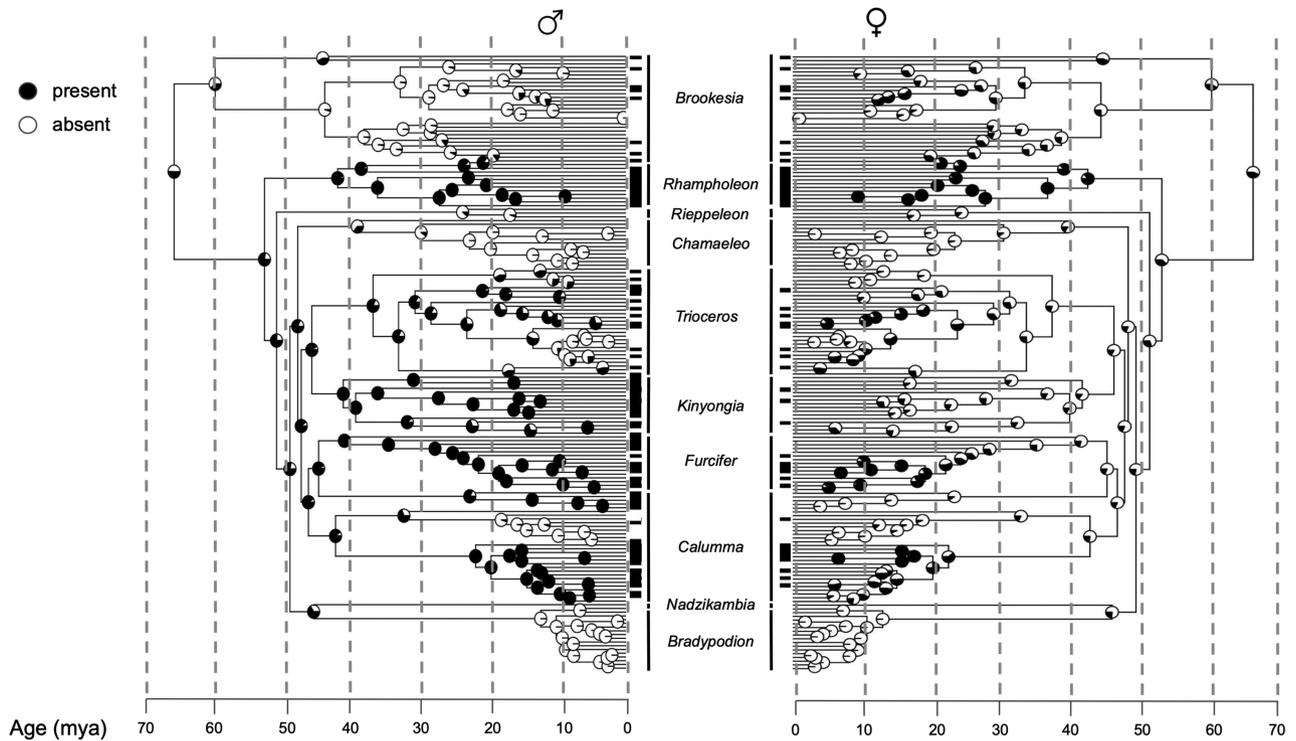
Figure 3. Evolution of rostral appendages in males (left) and females (right). Results shown are for the equal-rates (ER) model. Results for the all-rates-different (ARD) model are shown in Supplementary Figure S10. Proportional likelihoods of each state are shown in pie diagrams at each node (black = presence; white = absence). The black rectangles indicate that the appendages are present in extant species.

maximum ages of character gains in males predicted those of females. This regression was not phylogenetically corrected because the units of analysis were traits and not taxa. We used Welch's nonparametric two-sample *t*-tests (in R) to determine whether the two values (mean age, maximum age) differed between the sexes. We also performed linear regression to determine whether the number of character origins was associated with the maximum ages of characters. Here, origins referred to initial gains only, and not regains after loss.

Figure 4. Evolution of dorsal crests in males (left) and females (right). Results shown are for the equal-rates (ER) model; results for the all-rates-different (ARD) model are shown in Supplementary Figure S13. Proportional likelihoods of each state are shown in pie diagrams at each node (black = presence; white = absence). The black rectangles indicate that the crests are present in extant species.

For these analyses, we used the oldest node on which a trait was inferred to be present, but the trait could have evolved at any time along the branch between that node and the adjacent, older node (on which the trait was inferred to be absent). Using this oldest node gives a minimum estimate of the trait's age, but can systematically underestimate the ages of these trait origins. However, most of our analyses are based on comparisons among traits and sexes, and using the same approach for all traits and sexes should not bias these comparisons. Note that for trait gains along a terminal branch (extant species), we treated the age of origin as 0. This is consistent with our treatment of the time of origin of other gains (i.e., we are using the youngest node age for the branch on which the trait arose, not the oldest). The inferred ages of the origins of each trait using the maximum-likelihood reconstruction are given in Supplementary Dataset S4. The mean number and ages of the origins of traits using stochastic mapping are in Supplementary Dataset S5.

Hotspots and coldspots

To visualize hotspots and coldspots of weapon evolution, we first counted the total number of weapons present in each species. This was done separately for males and females. Hotspots were those parts of the phylogeny in which species had all or most weapon types present, whereas in coldspots, all or most weapon types were absent. To visualize the evolution of these hotspots and coldspots, we used the "contMap" function in the R package *phytools* (Revell, 2012). We estimated ancestral values of weapon number (in each sex) under maximum likelihood and visualized these changes along the branches of the tree. Note that this approach was only used for visualization, and not hypothesis testing.

To test whether hotspots and coldspots of weapon evolution were related to sexual size dimorphism or body size, we used the Poisson regression (Paradis and Claude, 2002) of the phylogenetic generalized linear model (phyloGLM) implemented in the R package *phylom* version 2.6.2 (Ho and Ané, 2014). The Poisson distribution is potentially appropriate when the dependent variable is a number (Paradis and Claude, 2002), such as the number of weapons analyzed here. We tested for a relationship between the total number of weapons (dependent) and maximum body size (SVL; independent) in each species and between number of weapons (dependent) and sexual-size dimorphism (SSD; independent) in each species. Analyses were performed separately for males and females. Since we lacked SVL data for both males and females for 32 species, we excluded these species from the analyses of SSD. We also excluded 17 species without data for males in the analysis of male size and weapon number. We pruned the excluded species from the treefile using the "drop. tip" function in *ape* version 5.7-1 (Paradis et al., 2004).

Relationships between weapons

To test for positive relationships between pairs of these binary characters (or trade-offs, negative relationships), we used phylogenetic logistic regression, utilizing *phylolm*. We looked for relationships between eight pairs of adjacent characters on the head and on the body. We adjusted these *p*-values for multiple comparisons using estimated false-discovery rates (FDR). This latter approach was implemented using the R package *FDRestimation* (Murray & Blume, 2021). The complete list of pairs of characters tested is in Supplementary Table S3 for males and Supplementary Table S4 for females. We predicted that some physically adjacent characters might show trade-offs (i.e., casques and rostral

appendages or casques and occipital lobes) due to differential energy allocation to one or the other character. For example, when characters are experimentally removed from insect bodies, adjacent characters attain larger sizes (Nijhout & Emlen, 1998). Furthermore, Emlen (2001) found that the production of horns in *Onthophagus* beetles results in smaller sizes of antenna, eyes, or wings. Alternatively, some pairs of characters may be positively associated. For example, in *Trioceros jacksonii*, the combination of three adjacent head characters seems to directly influence the outcome of intraspecific competition. Specifically, males with larger casques and horns have a significantly higher likelihood of winning intraspecific contests (Stuart-Fox et al., 2006; Van Kleeck, 2016). These three characters (rostral appendage, supraorbital spine, and casque) might therefore be positively related in their evolution among species. There are other pairs of body characters that are utilized in intraspecific fights (at least in some species) that may be associated with one another. For example, during the early stages of male–male combat, both dorsal and ventral spines and dorsal crests and dorsal spines become prominent during lateral compression in species such as *Trioceros jacksonii*, *Bradypodion pumilium*, and *Chamaeleo calyptratus* (Ligon & McGraw, 2013; Stuart-Fox et al., 2006; Van Kleeck et al., 2018). In addition, gular crests and gular spines are inflated in aggressive displays in both males and females (Stuart-Fox et al., 2006; Van Kleeck et al., 2018). These two characters might be associated among species.

Weapon evolution in males and females

We used phyloGLM to test whether the overall number of weapons in males (independent variable) predicted the number in females (dependent variable). Finally, we used phylogenetic logistic regression to test for associations between the presence of each weapon in each sex for all 11 traits. For each character, we used the male weapon as the independent variable and the female weapon as the dependent variable and adjusted all *p*-values for multiple comparisons using estimated FDR.

Note that for statistical analyses of the 10 stochastic mapping results, we used the *p*-values without adjusting for FDR. Instead, we focused on reporting the frequency of significant results among simulation replicates using the standard cutoff ($p < .05$).

Results

Patterns of gains and losses

The ancestral-state reconstructions for three select characters are shown in Figures 2–4 and for all other characters in Supplementary Figures S1–S8 (under the ER model). Reconstructions under the ARD model were not used, but are shown in Supplementary Figures S9–S19. Each weapon was gained multiple times (Table 2). The total number of gains for each character ranged from 4 to 20. For example, gular spines in males evolved 20 times. The number of losses for each character ranged from 1 to 16. Males had an average of 10.6 gains and 7.2 losses per character (mean among characters), a difference that was not statistically significant ($t = 1.46$, df = 18, $p = .159$). However, females exhibited significantly more mean gains (12.2) than losses (6.4; $t = 2.47$, df = 19, $p = .023$). Males and females did not differ significantly in their average number of gains (*t*-test: females = 12.2,

males = 10.6; $t = -1.75$, df = 20, $p = .464$) or losses (losses: females = 6.4, males = 7.2; $t = 0.31$, df = 20, $p = .757$).

A total of 100 stochastic character maps were generated for each character and sex and the summary maps are shown in Supplementary Figures S20–S41. These summary maps were very similar to the maximum-likelihood reconstructions. When analyzing gains and losses among the 10 stochastic simulations (Supplementary Dataset S5), males had an average of 13.6 ± 5.6 gains and 12.7 ± 8.9 losses per character (mean among replicates and characters), whereas females had an average of 15.6 ± 6.9 gains and 9.5 ± 7.4 losses. There were no significant differences in gains and losses between males and females (Supplementary Tables S5–S6) nor between gains and losses in males (Supplementary Table S7), but three of the 10 replicates showed that females exhibited more gains than losses (Supplementary Table S8).

The number of gains and losses of each character was related to when they first evolved (from maximum likelihood; Table 2). Based on linear regression among weapons, those that originated earlier in the tree were lost more often than those that evolved more recently, leading to a positive relationship among weapons between the oldest origin of each weapon and the number of losses (males: $r^2 = .73$, $p < .001$; females: $r^2 = 0.52$, $p = .013$). For males, the number of origins of a trait was significantly, negatively related to its maximum age, with older characters having fewer origins than younger characters ($r^2 = .48$, $p = .019$). This relationship was not significant in females ($r^2 = .06$, $p = .457$).

Results from stochastic mapping showed broadly similar patterns (Supplementary Tables S10–S13). There was generally a positive relationship between the oldest origin and number of losses (significant in 8/10 replicates in males, 6/10 in females; Supplementary Tables S10–S11). The relationship between number of character origins and maximum character age was consistently negative in males (but only significant in 1/10 replicates; Supplementary Table S12), and was often negative (6/10 replicates) but never significant in females (Supplementary Table S13).

Weapon persistence

The estimated age of each gain of each weapon in each sex is shown in Supplementary Dataset S4. A summary of weapon gains, losses, and ages is provided in Table 2. The oldest gains were of rostral appendages and dorsal crests in males (Table 2), which originated at least 65 million years ago (Mya). The oldest trait in females was casques, which originated at least 48 Mya (Table 2). Results were broadly similar in the stochastic simulations (Dataset S5). Rostral appendages and dorsal crests were the oldest traits in males, originating 60.5 and 56.1 Mya (mean among replicates), whereas dorsal crests and casques were the oldest in females (51.4 and 49.7 Mya).

Using maximum-likelihood reconstructions, results of linear regression between males and females among characters showed that both maximum and mean ages of characters were significantly related between males and females (maximum age in millions of years: $r^2 = .64$, $p = .003$; mean age: $r^2 = .42$, $p = .026$). Maximum ages were also significantly related between males and females in 8/10 stochastic character simulations (Supplementary Table S14).

Six of the 11 characters (i.e., rostral appendages, supraorbital spines, dorsal crests, dorsal spines, tail crests, tail spines) originated first in males (mean = 43 Mya; Table 2), then subsequently evolved in females (mean = 15 million years later;

Table 2. Estimated numbers of gains and losses of weapons in males and females. The number of origins includes only first-time evolutionary gains, not secondary gains that occur after losses. Total gains is the sum of origins and secondary gains. Total losses also include secondary losses after gains. Maximum age in millions of years is determined by the oldest gain or origin of a character. Mean age is calculated as an average of the ages at which each weapon is gained across all species in the phylogeny. The estimated ages are given in Supplementary Dataset S4.

Character	Origins	Secondary gains	Total gains	Total losses	Maximum age (Mya)	Mean age (Mya)
Males						
Casque	4	4	8	14	48	12
Rostral appendage	1	12	13	13	65	5
Supraorbital spines	9	0	9	3	35	12
Occipital lobe	4	0	4	6	31	24
Dorsal crest	1	5	6	16	65	11
Dorsal spines	8	7	15	16	48	9
Gular crest	13	0	13	1	30	7
Gular spines	20	0	20	3	30	6
Ventral spines	11	0	11	1	30	4
Tail crest	5	1	6	5	36	7
Tail spines	12	0	12	1	8	1
Females						
Casque	3	5	8	21	48	16
Rostral appendage	14	6	20	9	42	9
Supraorbital spines	10	0	10	5	32	10
Occipital lobe	5	0	5	8	31	19
Dorsal crest	8	3	11	9	43	29
Dorsal spines	12	4	16	9	28	7
Gular crest	13	0	13	1	30	7
Gular spines	20	0	20	3	30	6
Ventral spines	11	0	11	1	30	4
Tail crest	6	0	6	4	18	10
Tail spines	13	1	14	0	4	0

Table 2). The remaining five characters originated on the same branch for both sexes (mean = 34 Mya; Table 2). These included casques, occipital lobes, gular crests, gular spines, and ventral spines (Supplementary Figures S4–S6). Thus, none of the traits evolved in females before males. However, the mean maximum age of traits did not significantly differ between males and females (*t*-test: females = 30.5 Mya, males = 38.7 Mya; $t = 1.31$, df = 18, $p = .205$), nor did the mean ages of traits (*t*-test: females = 10.6 Mya, males = 8.9 Mya; $t = -0.57$, df = 19, $p = .577$).

Stochastic character maps also did not show a significant difference in maximum character ages between males and females in any of the 10 simulations (Supplementary Table S9). They showed that nine characters had older mean ages in males than females (especially rostral appendages, supraorbital spines, dorsal crests, dorsal spines, tail crests, and tail spines, differing by >5 million years or more; Supplementary Table S15). Two originated in females before males (casque, ventral crest), but the age differences were smaller (<4 million years).

Hotspots and coldspots of weapon diversity

We visualized hotspots and coldspots of weapon diversity as those parts of the tree where species had high or low numbers of weapons relative to other species (Figure 5; Supplementary Table S16). There were hotspots within the genera *Chamaeleo* and *Furcifer* (up to 7 weapons per species in males and females) and *Trioceros* (up to 10 weapons per species in males and females).

There were also coldspots of weapon diversity (Figure 5) in three genera (*Brookesia*, *Calumma*, and *Rieppeleon*). There were one or more species in these genera in which all weapons were absent (in both males and females). All weapons were also absent in females in one or more species of the genera *Furcifer*, *Kinyongia*, *Nadzikambia*, and *Rhampoleon*. These coldspots appear to have occurred at least partly through weapon loss, given that there were more weapons inferred to be present at the root than in some species in these genera (Figure 5) and based on reconstructions for individual variables.

In males (Table 3), the total number of weapons present in each species was significantly, positively related to SVL (phyloGLM: $n = 148$ species; $p < .0001$) and more marginally to SSD ($n = 133$; $p = .0402$; Table 3). In females (Table 3), weapon number was not associated with either SVL ($n = 133$; $p = .1185$) or SSD ($n = 133$; $p = .1835$).

Among the 165 sampled species, most (88%) had one or more of the 11 weapons. Widespread characters among species included rostral appendages (present in 94 species), casques (105 species), dorsal spines (105 species), and dorsal crests (115 species).

Relationships among weapons

Among the eight pairs of weapons compared using phylogenetic logistic regression, three pairs were significantly associated with one another in both males and females (Supplementary Tables S3–S4). Gular spines were significantly,
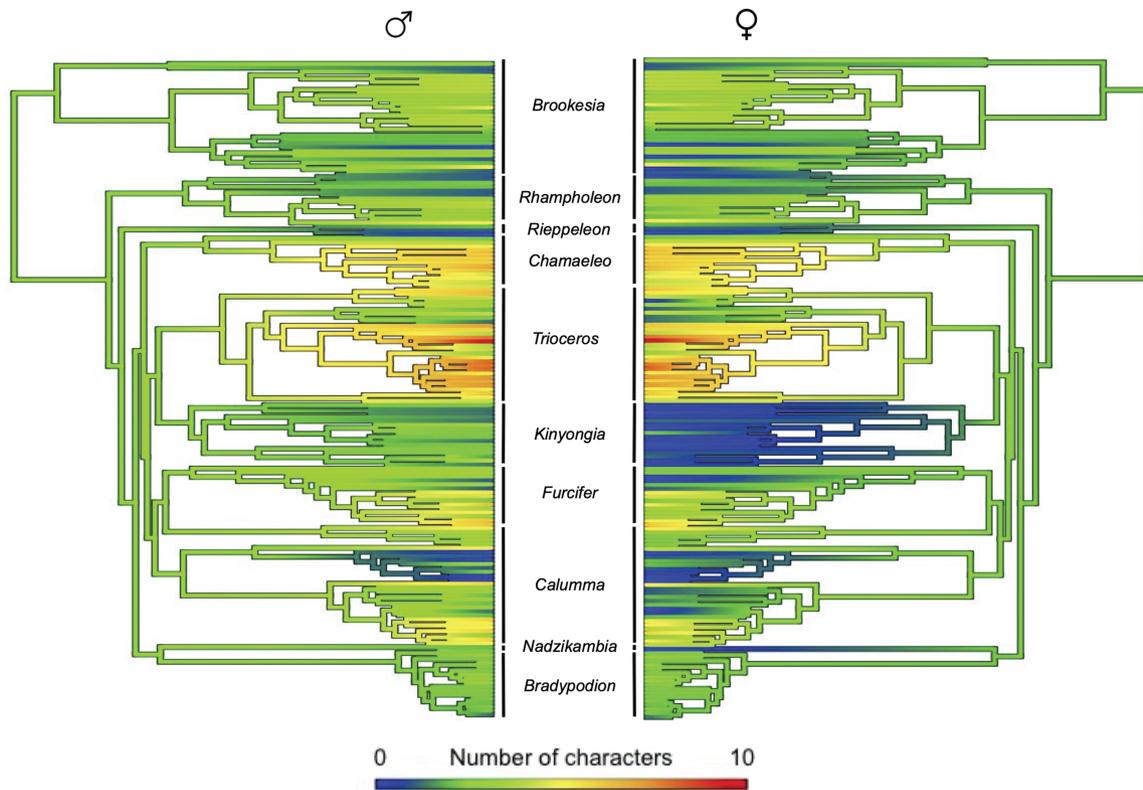
Figure 5. Total number of weapons in males and females. Cooler colors indicate low numbers of characters and warmer colors indicate increasing numbers of characters. Hotspots of weapon evolution occur within *Calumma*, *Chamaeleo*, *Furcifer*, and *Trioceros* and coldspots (all weapons absent in both sexes in one or more species) occur within *Brookesia*, *Calumma*, and *Rieppeleon*.

Table 3. Results of phyloGLM analyses between the numbers of weapons in each sex, body size (SVL), and degree of sexual size dimorphism (SSD). The sample size is $n = 148$ species for the first row and $n = 133$ species for all others. All *p*-values are adjusted for multiple comparisons using FDR and resulting significant values ($P < 0.05$) are boldfaced.

Dependent	Independent	Coefficient	Error	z	p
Number of male characters	Male SVL	0.0026	0.0007	3.8859	**.0004**
Number of male characters	SSD ratio	0.2209	0.0950	2.3252	**.0402**
Number of female characters	Female SVL	0.0013	0.0008	1.5611	.1579
Number of female characters	SSD ratio	0.1469	0.1098	1.3391	.1805

positively associated with gular crests (phylogenetic logistic regression: males: *p* < .001, females: *p* < .001), tail spines with tail crests (males: *p* < .001; females: *p* < .001), and ventral crests with dorsal crests (males: *p* = .0452; females: *p* = .019). Among the remaining pairs, we found two significant positive associations in males but not females: between dorsal spines and dorsal crests and between dorsal crests and ventral crests (Supplementary Tables S3–S4). We found a significant negative association between supraorbital spines and casques, but only in males. There were no significant associations between the remaining three pairs (tail crests and dorsal crests, supraorbital spines and rostral appendages, occipital lobes and casques).

Relationships between male and female weapon diversity

The total numbers of weapons present in males and females (Table 4; Supplementary Table S16) were strongly and positively related to each other among species (phyloGLM: *n* = 165, *p* < .001). These were also related when weapons

on the body (*n* = 5) and head (*n* = 6) were analyzed separately (phyloGLM: body: *p* < .001; head: *p* < .001; Table 4). However, the numbers of weapons present on the head and body were not strongly associated in males (phyloGLM: *p* = .863) nor females (*p* = .083; Supplementary Table S17). Individually, the presence of each weapon type was significantly and positively associated between males and females, based on phylogenetic logistic regression (Supplementary Table S18).

Discussion

Sexual selection is a major topic in evolutionary biology, but the large-scale macroevolution of weapons (i.e., traits used in male–male competition) remains little explored. Here we have posed and addressed several general questions about the evolution of sexually selected weapons, using chamaeleonid lizards as a model system. We describe and discuss these patterns below.

Table 4. Results of phyloGLM analyses of relationships between the number of weapons in males and females in each species. Results are shown for all weapons ($n = 11$) and for those on the body ($n = 5$) and the head ($n = 6$). All p-values are adjusted for multiple comparisons using FDR and resulting significant values ($p < .05$) are boldfaced.

Dependent	Independent	Coefficient	Error	z	p
Female characters	Male characters	0.1895	0.0133	14.292	**<.0001**
Female body characters	Male body characters	0.2634	0.0242	10.8875	**<.0001**
Female head characters	Male head characters	0.5931	0.0657	9.0296	**<.0001**

Major patterns of weapon macroevolution

Weapons can be gained and lost frequently

We found that the patterns of gains and losses of weapons varied from character to character. Nevertheless, we found that in males, every weapon character is gained at least four times and lost at least once. Some characters showed many gains (>10), including gular spines, gular crest, ventral spines, and tail spines, and many showed similar numbers of gains and losses. An earlier review (Wiens 2001) emphasized that losses are frequent in sexually selected traits, but did not explicitly compare the frequencies of gains and losses, and did not focus on weapons per se.

Our results suggest that the balance between gains and losses may be influenced by when traits evolve: older traits generally showed more losses and traits that have evolved only recently showed multiple gains. These relationships are not entirely unexpected: a trait that evolves deep in a phylogeny may have more opportunities to be lost than one that evolves more recently. Similarly, a trait that evolves deep in the phylogeny (without being lost) may leave fewer opportunities for the trait to be gained recently and repeatedly.

The repeated origins of each weapon are somewhat surprising and suggest that there may be similar selective pressures driving their repeated origins across the tree (and possibly similar genetic mechanisms leading to similar structures). This is surprising because some of these traits are largely unique among lizards (i.e., casques, rostral appendages, occipital lobe, supraorbital spines, ventral spines), but nevertheless evolved again and again within chameleons. Yet, Emlen et al. (2005) also found a similar pattern of repeated weapon gains. They examined the evolution of five different horn types (i.e., horns in different anatomical locations) among 48 species of the dung beetle genus *Onthophagus*. In males, they found that the oldest horn type (vertex) evolved once early in the history of the clade and was lost nine times (and regained once), whereas the other four horn types evolved more recently, and each had two or more gains and no losses (frons = 2 gains; clypeus = 2; medial pronotum = 9; lateral pronotum = 2). In females, they found one or more origins of each horn type (vertex = 3; frons = 2; medial pronotum = 7; lateral pronotum = 1), except the clypeus (0 gains). These results differ from ours in showing fewer losses, but are consistent in showing repeated gains (and fewer losses) among weapons that evolved more recently as opposed to deeper in the tree. Ord and Stuart-Fox (2006) examined patterns of evolution in seven potentially sexually selected traits in the sister group to chameleons (agamid lizards). Some of the traits they examined were similar to or overlapped with those examined here (e.g., rostral appendage). They found that five of these seven traits each had >20 gains across the tree in each sex, with fewer losses than gains in six of seven traits, but with at least one loss in all seven traits. These results suggest

that the repeated origins (and losses) of weapons may be a frequent pattern in animals.

Losses of weapons might occur when selection on these traits is relaxed (Lahti et al. 2009). Losses of sexually selected traits in general might be the result of environmental factors such as limited resource availability, social factors (e.g., declining female choice), or random factors like genetic drift (reviewed in Wiens, 2001).

Habitats can also influence the evolution of sexually selected traits. For example, Stuart-Fox and Ord (2004) found that agamid lizards occupying complex arboreal habitats had more characters (such as ridges, spines, gular pouches, and rostral appendages) than those in open habitats. In agamid lizards, these characters are also associated with aggressive territorial displays (Ord et al., 2015). Conversely, Bickle and Losos (2002) found larger weapons, such as casques and rostral appendages, in terrestrial chameleons as opposed to those occupying arboreal habitats. They suggested that larger structures may impede movement in arboreal habitats. However, our focus here was on the gains and losses of these traits, and not their size. Overall, these previous studies yield conflicting predictions about whether habitat might be important in the loss of weapons in chameleons, but there is evidence that habitat might be important for the loss of sexually selected traits in general (review in Wiens, 2001).

Weapons can be relatively old

Based on the ancestral-state reconstructions, 10 of the 11 weapons examined here were estimated to be at least 30 million years old (at least in males). The one exception had a maximum age of 8 Mya. Two characters each had a maximum age of 65 Mya. Results were similar using stochastic mapping (Supplementary Table S15), with all characters at least 10 Myr old, and eight >30 Myr old. These results suggest that weapons can be older than species or even genera and that large-scale phylogenetic analyses may be necessary to understand their evolution. Most importantly, it cannot simply be assumed that weapons are young (i.e., as old as single species).

Clades can contain hotspots and coldspots of weapon diversity

Our results identified hotspots of weapon evolution in which most weapon types were present (e.g., within *Trioceros* and *Chaemeleo*) and coldspots in which all or most were absent (e.g., within *Brookesia*, *Calumma*, and *Rieppeleon*). We found that most species had a low to intermediate number of weapons and that this was most likely the ancestral condition in the family (Figure 5). Intriguingly, our results suggest that all coldspots were achieved through losses of one or more weapons (Figure 5). We also found that hotspots were strongly associated with larger body size and more weakly associated with sexual-size dimorphism.

We are not aware of previous studies that have documented such hotspots for weapon evolution. However, Emlen et al. (2005) suggested that there was an overall pattern of weapon escalation in dung beetles, with some species having all five horn types examined and with most types showing only gains and no losses. Here, we show both escalation and de-escalation across different clades.

What might explain these hotspots? We found that the number of weapons in males was positively related to body size. Large male size in lizards is often attributed to intrasexual selection on behaviors such as aggression and territoriality (e.g., Cox et al., 2003). Additionally, larger organisms may have more energy to allocate toward sexually selected structures (e.g., Cornwallis & Birkhead, 2007; Gates et al., 2016). Thus, there is potential support for the idea that hotspots should occur in clades in which males are relatively large.

There can be positive relationships among different pairs of weapons in the same clade

We examined 8 pairs of weapons and found evidence for significant, positive relationships in three pairs of weapons in both males and females. The presence of multiple sexually selected signals has been documented in some species (e.g., Andersson et al., 2002; Moller & Pomiankowski, 1993). However, there have not been similar analyses of weapons per se (to our knowledge). Emlen (2001) documented trade-offs between the sizes of horns in different locations on the heads of beetles. We did find a significant negative relationship between one pair of physically adjacent traits in males (supraorbital spines and casques), but not in other pairs. The four pairs of traits with positive relationships in males (dorsal spines and crests, ventral and dorsal crests, gular crests and spines, and tail crests and spines) are all involved in lateral displays during intraspecific combat between males (Ligon & McGraw, 2013; Stuart-Fox et al., 2006; Van Kleeck et al., 2018). Therefore, they may be linked functionally in their evolution, but these traits can also evolve independently (e.g., gular spines often occur without crests and tail crests sometimes occur without spines). Our documentation of hotspots in weapon evolution in chameleons (by itself) suggests that there are not widespread trade-offs among different weapons in chameleons. However, it is important to note that our study primarily focused on the presence and absence of different weapons, and not on their size, and somewhat different patterns might emerge from considering the size of adjacent weapons as well.

Weapon evolution can be strongly correlated between males and females

There is a large literature that has compared the evolution of sexually selected traits in males and females (e.g., Baker & Wilkinson, 2001; Burns, 1998; Ord & Stuart-Fox, 2006; Wiens, 1999). However, this literature has rarely focused specifically on weapons (but see, e.g., Bro-Jørgensen, 2007; Emlen et al., 2005). Overall, we found that weapons were generally strongly related in their evolution between males and females across chameleons. For example, we found that the overall number of weapons was tightly related between males and females. Furthermore, when we analyzed each weapon separately using phylogenetic logistic regression, we found strong relationships between males and females for all of them.

Why do females have weapons at all? There are two main hypotheses to explain the presence of sexually selected traits in females (i.e., for those traits primarily associated with male-male competition or female choice). First, studies in mammals, insects, and birds have suggested that the presence of these traits in females is simply a genetic by-product of their presence in males (Baker & Wilkinson, 2001; Kraaijeveld, 2014; Kraaijeveld et al., 2007; Ord & Stuart-Fox, 2006; Potti & Canal, 2011). Alternatively, female traits may be under selection themselves, including directional selection for access to reproductive resources through competition with other females (Robison & Kruuk, 2007; Watson & Simmons, 2010), or male mate selection (Stuart-Fox & Goode, 2014; Weiss & Dubin, 2018). These two hypotheses may not be mutually exclusive (Amundson, 2000; Ord & Stuart-Fox, 2006). However, few studies have compared macroevolutionary patterns of weapon evolution among species between males and females (e.g., Emlen et al., 2005).

The genetic correlation hypothesis suggests that male and female traits originate together and then are either lost in females (yielding sexual dimorphism) or maintained through selection (Darwin, 1871; Lande, 1980; Lande & Arnold, 1985). We found only mixed support for this hypothesis. We found that weapons are highly correlated between males and females, potentially supporting the genetic correlation hypothesis. For example, 5 of the 11 characters originated on the same branch in both males and females and were largely maintained in females with few subsequent losses (based on ancestral reconstructions). However, the remaining six weapons evolved first in males, followed by their evolution in females ~15 million years later (on average). This pattern is not consistent with the genetic correlation hypothesis (as strictly defined above). Nevertheless, the presence of these weapons in females might still be related to having evolved first in males (through genetic mechanisms or otherwise), since we found strong relationships between the presence of weapons in males and females for all traits.

The separate origins of some of these weapons in females raise the question: what do females use these weapons for? Studies in other organisms suggest that females may evolve sexually selected signals or weapons given intrasexual competition for resources (e.g., Clutton-Brock, 2007, 2009), male preferences (e.g., Cornwallis & Birkhead, 2007), or even species recognition (Rand, 1961). Unfortunately, relevant behavioral studies involving female chameleons are lacking. However, data on movements suggest that home ranges of female Jackson's chameleons, *Trioceros jacksonii*, show significant overlap with one another, whereas males occupy distinct territories (Chiaverano et al., 2014; Van Kleeck et al., 2018). These data suggest that females may not vigorously compete for territories or resources at this spatial scale, at least in this species. Additionally, although mate selection by females is common and male chameleons of many species have the ability to assess reproductive receptivity of females, there is no evidence to suggest that male chameleons select females based on reproductive fitness (Karsten et al., 2009; Keren-Rotem et al. 2006). However, these studies only involve a few of the >200 known chameleon species (Stuart-Fox, 2014).

Areas for future research

Following from our findings, we see four obvious areas for future research. First, the patterns found here should be tested in other groups of animals, to address their generality. We suspect that the limiting factor for such analyses may be well-sampled time-calibrated phylogenies in those groups

with a diversity of weapons. Second, future studies on chameleons should further address the causes of these patterns. Further analyses of the function of these weapons in diverse chameleon taxa would be especially valuable. Our information on what these traits are used for comes from a very limited set of species at present. It is particularly unclear to what extent these traits are actually used in physical combat between males as opposed to signals in male–male competition (Table 1). Many traits might also be under sexual selection through female choice in addition to male–male competition (e.g., Karsten et al., 2009). The function of these traits might also vary across the tree. Third, additional analyses on the correlates of the gains and losses of these weapons would be useful, including variables related to habitat and social systems (e.g., Emlen et al., 2005). Fourth, our analyses are based on the presence and absence of these broadly defined traits. Future studies should also explore the detailed variation in morphology of these weapons, especially between males and females.

Conclusions

Sexually selected traits are a major focus of evolutionary studies, but large-scale macroevolutionary patterns in weapons remain largely unstudied. In this study, we analyze these patterns using chameleons as a model system. We found that most weapons were gained and lost repeatedly, with the balance of gains and losses generally related to when each weapon evolved (more losses in weapons that evolved earlier, more gains in weapons that evolved later). We found that many weapons were relatively ancient, with some as old as 65 million years (or more) and most older than 30 million years. We identified both hotspots and coldspots of weapon evolution, with some species in the hotspots having evolved almost all weapon types present across the family and coldspots in which all weapons were absent (mostly through secondary loss of weapons). We found limited evidence of trade-offs between adjacent weapons and instead found some pairs of weapons that were positively related. We also found that weapon evolution was strongly correlated between males and females, with weapons tending to evolve together in both sexes or in males first followed by females. These results provide a crucial baseline for comparison to weapon evolution in other groups and for comparisons to the evolution of sexually selected traits evolving under female choice.

Supplementary material

Supplementary material is available online at *Evolution*.

Data availability

All data and R code are included as Supplementary Materials (Datasets S1–S5), and are also available on Dryad (doi. org/10.5061/dryad.f7m0cfz2g).

Author contributions

M.V.K.H. and J.J.W. conceptualized the study. M.V.K.H. performed data collection and analyses. M.V.K.H. and J.J.W. wrote the manuscript.

Conflict of interest: The authors declare no conflict of interest.

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