Is diversification in male reproductive traits driven by evolutionary trade-offs between weapons and nuptial gifts?

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Many male animals have evolved exaggerated traits that they use in combat with rival males to gain access to females and secure their reproductive success. But some male animals invest in nuptial gifts that gains them access to females. Both these reproductive strategies are costly in that resources are needed to produce the weapon or nuptial gift. In closely related species where both weapons and nuptial gifts are present, little is known about the potential evolutionary trade-off faced by males that have these traits. In this study, we use dobsonflies (order Megaloptera, family Corydalidae, subfamily Corydalinae) to examine the presence and absence of enlarged male weapons versus nuptial gifts within and among species. Many dobsonfly species are sexually dimorphic, and males possess extremely enlarged mandibles that they use in battles, whereas in other species, males produce large nuptial gifts that increase female fecundity. In our study, we show that male accessory gland size strongly correlates with nuptial gift size and that when male weapons are large, nuptial gifts are small and vice versa. We mapped weapons and nuptial gifts onto a phylogeny we constructed of 57 species of dobsonflies. Our among-species comparison shows that large nuptial gift production evolved in many species of dobsonfly but is absent from those with exaggerated weapons. This pattern supports the potential explanation that the trade-off in resource allocation between weapons and nuptial gifts is important in driving the diversity of male mating strategies seen in the dobsonflies, whereas reduced male–male competition in the species producing large spermatophores could be an alternative explanation on their loss of male weapons. Our results shed new light on the evolutionary interplay of multiple sexually selected traits in animals.

1. Introduction
Male weapons are known to be important in male–male competition over access to mates. From the exaggerated antlers of deer to the enlarged mandibles of stag beetles, these condition-dependent traits grow largest in the best conditioned or highest quality males [1]. In mating systems where male weapons have evolved, the degree of exaggeration is assumed to be costly to the individual to produce such that investment of resources into the exaggerated trait comes at the expense of the growth of other morphological structures [2,3]. For example, the relative sizes of weapons in rhinoceros beetles (horns) and stag beetles (mandibles) are negatively correlated with the relative size of wings [4,5] such that allocation of resources to weapons reduces the growth of wings. Another example is from the flour beetle Gnatocerus cornutus which has sexually dimorphic enlarged mandibles. Artificial selection for increased relative mandible size in males reduced relative wing size, whereas artificial selection for decreased relative mandible size resulted in increased relative wing size [6,7]. Importantly, these morphological trade-offs also resulted in functional costs as those males with the largest mandibles, but smallest wings had the lowest dispersal frequencies [7].
Sperm competition theory predicts that there should be evolutionary trade-offs between energetically expensive traits and testes [8]. Recent studies have indicated that the enlargement of weapons, such as horns, mandibles or the head, reduces the relative sizes of the genitalia and testes or the number of sperm transferred to the female at mating [3,7–10]. But, what about species that have multiple mating strategies including premating competition and female choice?

Dobsonflies (Corydalidae: Corydalinae) belong to the holometabolous insect order Megaloptera. Adults of many species of dobsonfly are very large with wingspans frequently over 150 mm, but probably their most conspicuous feature are their sexually dimorphic enlarged mandibles [11,12]. Darwin [13] noted that male dobsonflies in the genus *Corydalus* (figure 1a) have enlarged mandibles much like stag beetles as a weapon for attacking rival males before mating, which we also directly observed (electronic supplementary material, movie S1). The enlarged head flanges in males of the genus *Platyneumus* (figure 1c) are another sexually dimorphic trait that is predicted to act as a male weapon although no observation on how flanges are used by fighting males is available. Thus, dobsonfly species exhibit a diversity of phenotypes from those that possess exaggerated mandibles such as males in the genera *Corydalus* and *Acanthacorydalis* (figure 1b) to males in the genus *Platyneumus* which have enlarged flanges on their head (figure 1c) but possess normally sized mandibles (figure 1c,d) to those that are not sexually dimorphic for any trait [11].

Not only do male dobsonflies have exaggerated, sexually selected traits, in some species, they also produce nuptial gifts [14]. Nuptial gifts in the form of spermatophores are common among the Megaloptera with many species attaching a sperm packet to the female genitalia which account for approximately 0.5–4% of the male’s total body weight [15,16]. However, *Protohermes grandis* which lacks enlarged mandibles produces a large, gelatinous spermatophore that they attach to the female genitalia during copulation [14]. These spermatophores are costly to produce as they comprise 10–20% of the male’s total body weight [17]. These nuptial gifts, eaten by the female after mating, contribute valuable resources to her and are widespread among Japanese *Protohermes* species [17]. Females and males benefit from this gift, as female *Pr. grandis* exhibit increased lifetime fecundity with multiple mating, suggesting that female consumption of nuptial gifts positively influences reproductive output [18].

Dobsonflies are uniquely suited to test the developmental allocation trade-off between weapons and nuptial gifts because they appear to have experienced significant selection for investment into both traits. To the best of our knowledge, this is a characteristic that is unique to dobsonflies. As a first step towards understanding how these traits arise and are maintained, we looked for patterns of male investment in exaggerated mandibles and nuptial gifts across 57 species of dobsonfly in a phylogenetic framework. First, we experimentally confirmed nuptial gift giving in two species that exhibit extreme differences in nuptial gift size by fluorescently tracing the path of male spermatophores into female ovaries. We then conducted a comparative analysis to examine relative weapon size and spermatophore size to determine whether or not the resource allocation trade-off predicts the evolutionary diversification of enlarged mandibles and nuptial gift giving in these insects. Finally, we mapped enlarged male mandibles and nuptial gifts onto our 57 species dobsonfly phylogeny and show a pattern that investment is either into weapons or nuptial gifts but never both.

2. Results

(a) Large nuptial gifts contribute to egg production in dobsonflies

Rhodamine B, a fluorescent thiol-reactive dye that forms a stable covalent bond with proteins, has been used to determine the ultimate fate of components of spermatophores [19]. In our

Figure 1. Male dobsonflies with large weapons have small accessory glands. (a) *Corydalus cornutus*, (b) *Acanthacorydalis fruhstorferi*, (c) *Neoneuromus maclachlani*, (d) *Protohermes grandis* and (e) *Platyneumus honduranus* (only the male head is shown). Note that the species with enlarged mandibles have smaller accessory glands. AG, accessory glands; FL, flange length; HW, head width; ML, mandible length; PTL, prothorax length; WL, forewing length. Scale bar, 10 mm.
study, rhodamine B was fed to males, and its passage during mating to females was compared between Pr. grandis and Parachauliodes continentalis, two species of Corydalidae. Male Pr. grandis (subfamily Corydalinae) possessed large internal reproductive organs consisting of several accessory glands and a pair of seminal vesicles (figures 1d and 2b right) and transferred a large spermatophore with a yellowish gelatinous substrate to the mated female (figure 2a, right). The spermatophore was eaten by the female after mating (electronic supplementary material, figure S4f). By contrast, Pa. continentalis (subfamily Chaulioidinae) had small internal reproductive organs (figure 2b, left) and transferred only a small sperm package to the mated female (figure 2a, left). Females of both species had immature eggs at emergence, which subsequently developed into mature eggs. The rhodamine-fed unmated females incorporated the dye into the developing eggs in the ovarioles (electronic supplementary material, figure S1); if fed with rhodamine later, after emergence, the dye was allocated more to the second row of eggs (electronic supplementary material, figure S1c,f). This independently confirmed that females use nutrients from their diet towards egg development.

No significant fluorescent signals were detected in females of Pr. grandis (n = 3) mated once with rhodamine-free (control) males (figure 2e). However, females singly (n = 4) and multiply (n = 2) mated with rhodamine-fed males showed fluorescent signals at the end of the first row of eggs in the ovarioles (figure 2f). This part of the ovariole includes nurse cells involved in the transportation of nutrients for egg development [20]. Stronger fluorescence was obtained in the other two females multiply mated with rhodamine-fed males; a weak but significant red signal was seen in each egg of the first row as well as at the end of the row of mature eggs (figure 2g), suggesting that the male nutritional gift is more significant when females have mated multiple times. By contrast, the small spermatophores that male Pa. continentalis transferred to females were not detected in females singly (n = 4) or multiply (n = 3) mated with rhodamine-fed males (figure 2c,d).

(b) Accessory gland size corresponds with the size of the nuptial gift

We measured the size of male internal reproductive organs relative to overall body size to predict nuptial gift size. We found that a fully charged accessory gland, indicated as a percentage of body weight, was very small in dobsonfly species of the genera Acanthacorydalis, Corydalus and Chloronius, whereas it was larger in species of the genera Neoneuromus, Neurhermes and Protohermes (figures 1 and 3). In order to estimate the average spermatophore size, the male internal reproductive organ size was measured for unmated (UM), just mated (JM) and recovering males (M), although the data were limited (electronic supplementary material, figure S3). The expected spermatophore size is thus large in species with large accessory glands (the differences between the values of UM and JM at the first copulation, and between the values of M and JM at the remating). To more fully explore this relationship, we observed spermatophores attached just

Figure 2. Variation in nuptial gift size (spermatophores) is observed to correspond to male accessory gland size, and this variation extends to the male contribution to egg production in Parachauliodes continentalis and Protohermes grandis. (a) A small spermatophore produced by male Pa. continentalis (left) and a large spermatophore with yellowish gelatinous substrate produced by male Pr. grandis (right). (b) Light (top) and fluorescence (bottom) photographs of male internal reproductive organs excluding the testes of rhodamine-free and rhodamine-fed Pa. continentalis (left two) and Pr. grandis (right two). Red fluorescence in rhodamine-fed males of each species. (c,d) Ovarioles of female Pa. continentalis mated once with rhodamine-free (control) males (e) and those mated once or three times with rhodamine-fed males (f,g). In (f), fluorescent signals were detected at the end of a row of mature eggs (indicated by arrowheads). In (g), the fluorescent signals were also detected in each egg of the row of mature eggs.
after copulation of eight species that we could collect and maintain in the laboratory. We found that *Neuvromus gloriosai*, *Neuhermes sumatrensis*, *Protomonis sabaeiensis*, *Protomonis costalis*, *Protomonis immaculatus* and *Pr. grandis* had relatively large spermatophores (electronic supplementary material, figure S4) which corresponded to their large accessory glands relative to overall body size (electronic supplementary material, figure S3). In *Corydalus cornutus* and *Corydalus luteus*; however, only a small sperm packet without a gelatinous mass was attached to the female genitalia, and it could hardly be seen from the outside even just after mating (electronic supplementary material, figure S4). We were not able to examine the accessory glands of *Chloroniella* (South Africa) or *Platyneuromus* (Central America) species because of their extremely narrow distribution in areas where we were unable to collect live insects. Our sample sizes were extremely low owing to the difficult nature of collecting mating pairs and observing matings in the laboratory; therefore, we report no data in this section besides the observational data that we show. Nevertheless, we speculate that because all appendages of the male genitalia of *Chloroniella* have been measured and found to be smaller and shorter than those of other genera [21], males of this genus probably transfer small spermatophores. In *Platyneuromus*, males are predicted to transfer a small spermatophore based on the simple male genital structure that has been reported [22]. In fact, the spermatophore has been reported to be invisible on the female abdominal terminalia just after mating in *Platyneuromus soror* providing further evidence of little to no nuptial gift giving in this genus [23]. Our data on male accessory gland size and relative spermatophore size indicate that nuptial gift donation is greater in *Neuvromus*, *Neuhermes* and *Protomonis* than in the other genera (figure 3).

**Figure 3.** Allometric relationships of traits in dobsonflies. Body, weapon, head and wing sizes were calculated as the relative difference between the male and female means: (male mean—female mean)/female mean. For weapon, head, and wing sizes, the adjusted means were calculated from the male and female regressions (see also the electronic supplementary material, figure S2). Asterisks indicated for the body size bars indicate a significant difference between the male and female means by Student’s *t*-test (*p* < 0.05), and asterisks for all other traits indicate no overlaps in 95% confidence intervals between male and female adjusted means. Nuptial gift size is shown as the per cent accessory gland weight/body weight ratio (bars, s.d.) of the males, excluding those that had just mated (see the electronic supplementary material, figure S3, dataset S1). *peri*, *peringueyi*; *unim.*, unimaculata; *orie.*, orientalis; *fruh.*, fruhstorferi; *soro.*, *soro*; *hond.*, honduransus; *mexi.*, mexicanus; *miri.*, mirifica; *lute.*, *luteus*; *flav.*, flavicanis; *corn.*, cornutus; *txa.*, texanus; *arma.*, armatus; *perv.*, peruvianus; *igno.*, ignobilis; *sp.*, species; *mac.*, macalachani; *exte.*, exterior; *glor.*, gloriosai; *suma.*, sumatorensis; *gran.*, grandis; *imma.*, immaculatus; *costal.*, costalis; *disj.*, disjunctus; *yang.*, yangi; *weel.*, weelei; *diff.*, *differentalis*; *saba.*, sabahensis; and *davi., davi*; ne indicates not examined.
Figure 4. Phylogeny of 57 dobsonflies (family Corydalidae, subfamily Corydalinae) based on the combined molecular + morphological data inferred from Bayesian analysis. This topology is congruent with the parsimony topology. Bootstrap supports ≥ 50 and Bayesian posterior probabilities ≥ 0.50 are given at each corresponding node. Besides Chloroniella, four major lineages are recovered, comprising the Protohermes lineage (Protohermes and Neurhermes), the Neoneuromus lineage (Neoneuromus and Neoneuromus), the Acanthacorydalis lineage (Acanthacorydalis only) and the Corydalus lineage (Platyneuromus, Chloronia and Corydalus). Lineages marked red exhibit male weapons and lineages marked blue produce large nuptial gifts. Evolutionary gains of large nuptial gifts (blue circle) and male enlarged mandibles (red square) across lineages, as well as secondary loss of large nuptial gifts (blue cross), are shown as supported by ancestral state reconstruction (electronic supplementary material, figure S5). Enlargement in male head flanges (red triangle) is unique to Platyneuromus. The outgroup taxa used here are the fishfly Protochauliodes incertus (family Corydalidae, subfamily Chauliodinae) and the alderfly Sialis kumejimae (family Sialidae) of the same Order Megaloptera.

supplementary material, figure S2). Male mandibles/head flanges were thus relatively larger than female mandibles/head flanges at $p = 0.05$ level in Acanthacorydalis, Platyneuromus and Corydalus, but one exception was Pr. costalis (figure 3). In these three genera with exaggerated traits, the adjusted mean of male mandible or head flange size was always significantly larger than in females (figure 3). In observations of mating with combinations of two males and one female, male Acanthacorydalis orientalis, C. cornutus and C. luteus used their mandibles to attack rival males, but never the female (electronic supplementary material, movie S1). Although the enlarged flanges in male Platyneuromus have not been examined with regard to their function, the observed positive allometries suggest that these organs are important for sexual selection.

Head width was a strong predictor of body size in both sexes, because the slope of the allometric regression to the prothorax length did not differ from 1 at $p = 0.01$ level in all species, excluding one exceptional C. cornutus in which a positive allometry suggests that male head size of this species is correlated with mandible size (dataset S1, electronic supplementary material, figure S2). In most species, the adjusted means of head width were similar between the sexes, except in Platyneuromus honduranus and Corydalus flavicornis where males had significantly larger heads than females and Acanthacorydalis unimaculata and Pr. grandis which had significantly smaller heads (figure 3).

Forewing length in many species of dobsonflies is sexually dimorphic. We found that forewing length exhibited a negative allometry slope in the three species of Acanthacorydalis, Pr. grandis and Pr. immunculatus (dataset S1, electronic supplementary material, figure S2). Forewing length was also shorter in males compared with females in most species of Acanthacorydalis, Corydalus, Neoneuromus, Neoneuromus, Neurhermes and Protohermes (figure 3). However, forewing length was not significantly different between the sexes in Chloroniella, Platyneuromus and Chloronia (figure 3). The difference in forewing size was significantly correlated with species that possessed either enlarged mandibles (Acanthacorydalis and Corydalus) or large nuptial gifts (Neoneuromus, Neoneuromus, Neurhermes and...
Protohermes) such that males that possessed either of these two traits has significantly shorter forewings than females (figure 3 and electronic supplementary material, figure S2).

(e) Evolution of male weapons and nuptial gifts

Parsimony and Bayesian phylogenetic analyses based on a combined dataset of 16S rRNA + COI + ND2 sequence data (for a total of 1862 bp) plus 71 morphological data revealed fundamentally consistent topologies, and the 50% majority-rule consensus tree from the Bayesian inference is presented in figure 4, with enlarged male weapons or nuptial gifts mapped onto the relevant lineages. The South African endemic genus Chloroniella, which represents the basal most dobsonfly in our study, has no weapons and is predicted to produce a small spermatophore. In the Asian genera Neurhermes and Protohermes (the Protohermes lineage), males are smaller than females and have no weapons as in Chloroniella, but produce a large spermatophore as a nuptial gift. The remaining genera consist of three lineages, the Neoneuromus lineage (Neoromas and Neoneuromus, endemic to Asia), the Acanthacorydalis lineage (Acanthacorydalis only, endemic to Asia) and the Corydalus lineage (Platyneuromus, Chloronia and Corydalus, endemic to the Americas). First, males of the Neoneuromus lineage have no weapons and have reduced body size, but they produce a relatively large nuptial gift. The Acanthacorydalis males show sexual dimorphism in body size and weapon size, but not in head size and their nuptial gift is small. In the Corydalus lineage, body size differences are small between the sexes. However, Platyneuromus males have exaggerated flanges and Corydalus males have exaggerated mandibles. The ancestral state reconstruction analyses we performed indicate that large male nuptial gifts were gained by most do bsonfly genera except for Chloroniella, but this trait was probably lost in the Acanthacorydalis and Corydalus lineages (figure 4 and the electronic supplementary material, figure S5a). In the latter lineages, enlarged male weapons have evolved in Acanthacorydalis (large mandibles), Platyneuromus (large head flanges) and Corydalus (large mandibles), but not in Chloroniella (figure 4). Enlarged male mandibles were gained independently in Acanthacorydalis and Corydalus (electronic supplementary material, figure S5b) and enlargement of the head flanges was unique to Platyneuromus.

3. Discussion

The phylogeny of the worldwide do bsonfly genera that we constructed combining molecular + morphological data is congruent with the latest morphological phylogeny [24], in which the South African endemic genus Chloroniella was assigned as a sister group with all the other do bsonfly genera. We also found that monophyly of the Protohermes lineage, the Neoneuromus lineage and the Corydalus lineage was well supported here as in the morphological phylogeny. However, Acanthacorydalis was placed as a sister lineage to the Corydalus lineage. Within the Corydalus lineage, Corydalus was assigned as a sister group of Chloronia, as in all former morphological phylogenies of Corydalinae [11,25], although Corydalus and Platyneuromus formed a weakly supported monophyletic clade in the morphological phylogeny of this subfamily reconstructed by Contreras-Ramos [24]. Within the Protohermes lineage, Protohermes was paraphyletic with Neurhermes, reducing support for its generic status and instead attributing it as a synonym of Protohermes.

The outgroup taxa of do bsonfly are alderflies (Sialidae) and fishflies (Corydalidae; Chauliodinae). In all species of alderflies and fishflies in which the mating behaviours have been examined to date, males transfer a spermatophore consisting of only the sperm package to the female and their internal reproductive organs are very small [15,16]. As confirmed in the fishfly Pa. continentalis in this study, their small sperm packages seem to contribute little nutrition to the mated female, even though the package is partially consumed by the female after mating. Thus, transfer of a small sperm package is the most parsimonious ancestral state, with the production and transfer of a large nutritious spermatophore as a derived character state, which is also supported by our ancestral state reconstruction analysis (electronic supplementary material, figure S5a). A large nutritional spermatophore is present in the Protohermes and Neoneuromus lineages, but is absent in the Acanthacorydalis and Corydalus lineages.

Sexual dimorphism in mandible and head shape are also derived trait states based on this phylogeny, as none of the outgroup taxa possess these [12,26]. Our phylogeny supports the independent evolution of male weapons in the do bsonfly lineage at least three times—in Acanthacorydalis, Corydalus and Platyneuromus. Alternatively, two instances of weapon development may have occurred in the Acanthacorydalis and Corydalus lineages (electronic supplementary material, figure S5b); however, Platyneuromus developed head flanges, whereas Corydalus developed mandibles, suggesting independent acquisition of such enlargement of male traits. The well-developed male mandible shapes differ between Acanthacorydalis and Corydalus. Acanthacorydalis males have mandibles with small teeth, but Corydalus males have cylindrical mandibles that lack teeth [12,25]. These weapons of male Acanthacorydalis, Corydalus and Platyneuromus are suggested to be sexually selected traits via male—male combat for mates. Indeed, the allometry between male body size and weapon size is positive (significantly greater than 1), and the adjusted means of male mandible or head flange sizes were always significantly larger than those of females.

In this study, we show that males in different do bsonfly lineages exhibit differences in the relative sizes of two different sexually selected traits—weapons used in male—male competition and nuptial gifts important for male reproductive success. The do bsonfly genera with enlarged weapons (i.e. Acanthacorydalis, Corydalus and Platyneuromus) tended to have relatively small nuptial gifts, whereas those without any sexual dimorphism in mandible shape (i.e. Neoneuromus, Neoromas, Neurhermes and Protohermes) tended to have relatively large nuptial gifts (figure 3). These observations support the hypothesis that competition in resource allocation exists between these two traits in do bsonfly species. In Chloroniella and Chloronia, males have no enlarged weapons and nuptial gifts. The former is the sister genus to all other do bsonfly genera and possesses neither weapons nor nuptial gifts which is the ancestral state reconstructed for do bsonflies. However, Chloronia belongs to the Corydalus lineage and may have secondarily lost large nuptial gifts and did not evolve weapons. To the best of our knowledge, no do bsonfly species has been described that exhibits both exaggerated weapons and large nuptial gifts. Our study relies on field collected individuals and not laboratory studies of resource allocation; therefore, the association between

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produces more offspring of better quality [33–36]. Thus, females become the competitive sex, and there is a reduction of male sexual competition as the operational sex ratio shifts from male-biased to female-biased. In dobsonflies, male mating frequency decreases in species which have large spermatophores [17], and remating by females is high during their lifetimes [18]. Therefore, an alternative explanation may also predict a reduction in the development of male weapons because of a reduction of sexual selection, particularly male–male competition in the species producing large spermatophores.

In summary, sexually selected traits not only exhibit evolutionary trade-offs with non-sexually selected traits such as wings, but also with costly behavioural traits and morphological traits associated with mate acquisition. Our results show that in dobsonflies, some lineages evolved large weapons, whereas others evolved large nuptial gifts with either no weapons, or small weapons relative to body size. Our data support the hypothesis that individuals invest in either weapons or nuptial gifts but not both, whereas the loss of male weapons in nuptial gift-giving dobsonflies could be due to relaxed selection on male–male competition. Our results from the world’s dobsonflies suggest that the evolution of costly sexually selected traits such as male weapons and nuptial gifts probably results from the interaction between nutritional ecology and life-history evolution and results in the remarkable diversity in shape and form in animals. Megaloptera represent an important new system to study the interplay among multiple sexually selected traits. It will be important to examine whether or not the lack of male weaponry in the nuptial gift-giving dobsonflies is due to limited resources, relaxed sexual selection or to other ecological factors in future studies, especially direct empirical studies on resource allocation of weapons and nuptial gifts.

4. Material and methods

(a) Specimens

Most specimens for morphological measurement and molecular phylogenetic analyses have been deposited in the Entomological Museum of China Agricultural University, Beijing, China (CAU) and Hayashi Collection in Tokyo Metropolitan University, Tokyo, Japan (HC). In addition, a few specimens for the morphological measurement were deposited in several other museum collections that are listed in the electronic supplementary material.

(b) Rearing and mating

The larval dobsonflies were collected with hand-nets from stony streams. Methods for rearing can be found in the electronic supplementary material. Matings were conducted by placing a male and a female prior to the dark period in individual cages (20 × 30 × 20 cm³) constructed of nylon netting. At 1 h intervals from darkness to the next light, insects were checked for their mating status under a dim red light. After copulation, individuals were returned to their respective rearing vessels. Individuals of both sexes were allowed to copulate only once. The numbers of mating bouts observed in the laboratory were deposited in several other museum collections that are listed in the electronic supplementary material.
were required for the insects collected for this study in China, Japan, Malaysia, Mexico and USA. The field studies were conducted in accordance with local legislation and did not involve any endangered or protected species.

(c) Rhodamine B treatment
Rhodamine B was traced from the male to the mated female between two species of Megaloptera with contrasting spermatophore shape, *Pr. continentalis* (Corydalidae: Chauliodinae) with a small spermatophore and *Pr. grandis* (Corydalidae: Corydalinae) with a large spermatophore. Adults were raised from field-collected final-instar larvae. Collecting information and measurements of adults of these two species can be found in the electronic supplementary material. Adults were given a sugar solution daily after emergence. Among them, several males were given a mix (1:5 by volume) of 0.00417 M rhodamine B solution (Wako Pure Chemical Industries, Tokyo, Japan) and sugar solution for two consecutive days, but thereafter, were given only the sugar solution daily. More than 3 days after the last rhodamine-containing feed, the rhodamine-fed males were used for mating trials. Two, four and three females of *Pa. continentalis* and three, four and four females of *Pr. grandis* were mated singly with rhodamine-free (control) males, singly with rhodamine-fed males, and multiply with rhodamine-fed males (three times with different males at 1 to 4 day intervals), respectively. Four days after the last mating, the females were anesthetized with ether and dissected to examine their ovarioles under a fluorescence microscope (BIOREVO BZ-9000; Keyence Corporation, Osaka, Japan). The male internal reproductive organs dissected out after anesthetization were observed under a fluorescence stereomicroscope (MZ FLIII; Leica, Wetzlar, Germany) with a green filter (excitation maximum 540 nm, emission maximum 625 nm). Unmated females given the rhodamine diet once in the early or late stage after emergence were also examined to determine the incorporation of nutrients from the diet into the developing eggs.

(d) Accessory gland size
Male body weight (BW, mg) was measured to the nearest 0.01 mg with a microbalance (Mettler AT201; Greifensee, Zürich, Switzerland) after they had been killed with ether. The internal reproductive organs were then dissected out and weighed immediately (AG, mg). When dissected out, testes and fine-tubed vas deferens were always cut from the remaining part (clump of accessory glands) of internal reproductive organs. The ratio of AG/BW (%) was calculated for each male. The ratios of AG/BW estimated based on the dry and fresh weight did not differ significantly [17]. Therefore, in this study, the ratio was based on fresh-weight measurements.

(e) Allometric relationships
Morphological measurements were made for a total of 1819 adult specimens. The head width, mandible (or flange) length, prothorax length and forewing length were measured using slide callipers to the nearest 0.05 mm (figure 1). Allometric relationships were quantified from log-transformed data regressions of each trait on the prothorax length as a body size indicator. When the slope of the regression line was greater than 1, 1 or less than 1, the trait was taken to show positive allometry, isometry and negative allometry, respectively, against body size [37]. The slopes were calculated by major axis regression for males and females, respectively, because the standard least-squares method tends to produce underestimations [38]. The adjusted means were calculated directly from the regression lines when the slopes of males and females differed at \( p = 0.05 \) on ANCOVA, but estimated from the regressions with the common slopes when they did not differ significantly. The 95% confidence intervals of each adjusted mean were calculated by the bootstrap method with 1000 replicates.

(f) Phylogenetic analysis
Fifty-seven dobsonfly species, representing nearly half of the world’s species, were selected for the present analysis, including representatives from all nine genera of Corydalinae worldwide (electronic supplementary material, table S1). We chose three mitochondrial genes, i.e. large ribosomal RNA gene (16S rRNA) and two protein-coding genes, cytochrome oxidase subunit 1 (COI) and NADH dehydrogenase subunit 2 (ND2), as the molecular markers. Protocols of DNA extraction and PCR can be found in the electronic supplementary material.

The sequences of the three markers were aligned using CLUSTALX [39] with default settings, whereas the alignment of 16S rRNA sequences was manually checked to improve the accuracy based on the published secondary structure for 16S rRNA of the fishfly species *Neochaolaides punctatodus* (Megaloptera: Corydalidae: Chauliodinae) [40], and the protein-coding gene sequences were aligned according to the translated amino acid codons. In addition, we coded 71 morphological characters obtained from the latest study on the morphological phylogeny of Corydalinae [24]: appendix 5. The morphological data were combined with the aligned DNA sequence data, being the dataset for the subsequent phylogenetic analyses (electronic supplementary material, dataset S2). Parsimony analyses were conducted using PAUP* 4.0 [41]. Bayesian analyses were performed using MrBayes 3.1.2 [42]. Detailed settings of tree reconstruction can be found in the electronic supplementary material.

(g) Ancestral state reconstruction
To test the evolution of the two sexually selected male traits across our phylogeny of dobsonfly genera, we performed an ancestral state reconstruction by using the program RASP2.0 beta [43]. We tested sexually dimorphic mandibles and nuptial gifts separately tested as independent characters, and the presence/absence of these two characters was used as the character state for each terminal taxon. The fully resolved interfamilial phylogeny of Corydalinae with binary topology was used for character mapping. A Bayesian binary Markov chain Monte Carlo analysis was run with the default settings. Proportional probabilities of each ancestral state were indicated at corresponding nodes.

Data accessibility. Our sequence data are available in the NCBI with the GenBank accession listed in the electronic supplementary material, table S1. Other dataset are available in doi:10.5061/dryad.4bd12.

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