Predator-Induced Tail Coloration Toward Diverse Dragonfly Nymphs in Tadpoles of the Japanese Tree Frog (*Dryophytes japonicus)*

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Abstract

Anuran larvae can change their coloration and morphology to avoid predation. Particularly, some tadpoles in the genus *Dryophytes* in the Americas develop deep tail fins and bright orange tail coloration in response to predators such as dragonfly nymphs. These conspicuous tails are hypothesized to attract predator attacks, protecting vital body parts from fatal injuries. However, it remains unclear whether the extent of the induced phenotypic changes differs between various predators with distinct feeding patterns and habitats. To explore this, we reared tadpoles of the Japanese tree frog, *Dryophytes japonicus*, with four dragonfly species, water bug, water scorpion, or newt, and measured phenotypic changes, especially focusing on tail coloration. We found that the aeshnid *Anax nigrofasciatus*, a sit-and-wait predator that climbs on aquatic vegetation, induced bright orange and broad tails of tadpoles. Other odonate species that forage in and on the substrate also elicited tail coloration or deeper larval tails, but it took more time to induce the tadpoles' response compared to *A. nigrofasciatus*. In contrast, non-odonate predators did not induce color and morphological changes. This study provides the first evidence of predator-induced tail coloration in an Asian species of *Dryophytes*, which appears to be a defensive response specifically to dragonfly nymphs, as visually adept predators. Moreover, the differences in the extent and timing of the phenotypic changes among odonate species suggest that anti-predator phenotypes are modulated based on relative predation risks, considering the predators' foraging patterns and visual capabilities, thereby allowing tadpoles to minimize physiological costs and unintended risks associated with the induced traits.

Introduction

The body color of organisms has diverse implications for predator–prey interactions. Many species exhibit cryptic body color, which suggests significant selective pressure from predators (Norris and Lowe, 1964). Cryptic coloration contributes to predation avoidance by representing background matching, countershading, and the disruption of body shape or outlines (Thibaudeau and Altig, 2012). In contrast, some organisms use bright colors and expendable body parts to divert predator attention away from more vulnerable areas (Hawlena et al., 2006). Consequently, body color contributes to predation avoidance by fulfilling either of two primary functions: enhancing prey crypticity or creating a defensive lure phenotype (Touchon and Warkentin, 2008).

Changes in body color are often induced by predators through phenotypic plasticity. The utilization of a prey's body coloration is highly dependent on its context, including the sensory capabilities of predators and the background color (e.g., Espanha et al., 2016). For example, although the poison dart frog (*Dendrobates tinctorius*) is well recognized for its typical aposematic signaling, its conspicuous coloration also functions as camouflage when viewed from a distance, blending with the background (Barnett et al., 2018). Especially in aquatic habitats, substantial variation exists in background conditions and the composition and abundance of predators across different localities, making it difficult for prey to achieve a uniform predation avoidance ability across their range (Van Buskirk, 2002; McIntyre et al., 2004). Therefore, such environmental uncertainty has generally been believed to favor plastic rather than constitutive anti-predator defenses (Agrawal and Karban, 1999; McIntyre et al., 2004).

Anuran larvae, or tadpoles, have served for decades as a model system for investigating induced defense mechanisms, as well as predator avoidance strategies and trade-offs mediated by plastic traits (Kasumovic, 2013; Kruger and Morin, 2020). Phenotypes such as body color and shape are generally subject to predator selection for survival and mate choice, but the latter can be ruled out in the case of tadpoles. They often rely on chemical cues

61 to evaluate predation risk through continually released predator-borne cues and digestion-
62 released, prey-borne cues, and then exhibit predator-induced defense mechanisms (Brönmark
63 and Hansson, 2000; Hettyey et al., 2010, 2015).

64 Predator-induced plastic responses in tadpoles can be categorized into four primary
65 domains: behavioral, morphological, physiological, and chemical defenses. Behavioral
66 responses encompass a reduction in overall activity (e.g., Lawler, 1989; Takahara et al.,
67 2012a), increased burst swimming speed (Takahara et al., 2008), and spatial avoidance of
68 predators (e.g., Laurila et al., 1998; Nunes et al., 2013). Morphological changes for predator
69 avoidance have been well documented in tadpoles of various species (Relyea, 2001; Van
70 Buskirk, 2002; Richter-Boix et al. 2007). For instance, tadpoles undergo changes in body size
71 (i.e., correlated with growth rate and metamorphosis timing) and shape, such as the
72 development of deeper tail fins (Lardner, 1998, 2000). Behavioral and morphological
73 responses are sometimes co-expressed (Hossie et al., 2017). Physiological responses include
74 changes in respiration rate and gut evacuation rate (Steiner, 2007; Steiner and Van Buskirk,
75 2009; Costello and Michel, 2013). These may arise due to trade-offs in predator avoidance
76 (Relyea and Auld, 2004) or as responses to partially offset the costs of lower energy intake
77 resulting from evasion (Barry and Syal, 2013). Additionally, tadpoles of certain groups,
78 particularly toads, evade predation through toxin production (Bókony et al., 2016; Hettyey et
79 al., 2019).

80 The types and intensities of induced responses in tadpoles often differ according to
81 predator types (Relyea, 2001; Teplitsky et al., 2004, 2005; Hettyey et al., 2011). For instance,
82 Kishida and Nishimura (2005) revealed that tadpoles of *Rana pirica* developed deeper tail
83 fins in response to dragonfly nymphs, whereas in the presence of salamander larvae, they
84 exhibited not only deeper tails but also bulging heads and bodies. Tadpoles expressing these
85 predator-specific traits should have a survival advantage over those with mismatched or non-
86 induced phenotypes when faced with predation. Conversely, expressing such defense traits
87 incurs energetic costs and can increase vulnerability to non-focal predators (Touchon and

Warkentin, 2008; Innes-Gold et al., 2019). Due to these costs, defense traits are expected to remain plastic rather than fixed, and their types and intensities can vary depending on predator species, sometimes exhibiting predator-specific responses.

Dragonfly nymphs, as one of the major predators of tadpoles, are known to induce morphological responses characterized by deep tail fins across many anuran species (Relyea and Werner, 2000; Van Buskirk, 2002). This induced broad tail has been repeatedly hypothesized to improve the swimming performance of tadpoles and facilitate their escape from predators (Smith and Van Buskirk, 1995; McCollum and Leimberger, 1997; Van Buskirk and McCollum, 2000a). However, experimental evidence of this benefit is lacking (Van Buskirk et al., 2003; Johnson et al., 2008). Moreover, a few tree frog species in the genus *Dryophytes* respond to odonate predators by not only exhibiting deeper tails but also turning their entire tails bright orange (McCollum and Van Buskirk, 1996; Van Buskirk and McCollum, 1999; LaFiandra and Babbitt, 2004). Thus, as another function, the induced deep and brightly colored fin is thought to serve as a lure, diverting predator attacks away from the more vulnerable head and body regions (Van Buskirk et al., 2003, 2004; Johnson et al., 2008). Given that tail fins tear easily (Doherty et al., 1998) and that tadpoles can maintain their swimming performance even after partial tail loss (Hoff and Wassersug, 2000; Van Buskirk and McCollim, 2000b), the tail is considered expendable relative to other body parts. Because dragonfly nymphs are predominantly visual predators, exhibiting the ability to discern both the shape and color of immobile as well as moving prey (Bybee et al., 2012; Espanha et al., 2016), color and shape changes in tadpoles are likely to be effective in avoiding predation by odonates.

Among dragonfly nymphs, several ecological types have been identified based on microhabitat usage and foraging behavior (Pritchard, 1965; Corbet, 1999), including climbers (e.g., Green darner, *Anax junius*), moving among aquatic vegetation and ambushing prey on plants; sprawlers (e.g., Globe skimmer, *Pantala flavescens*; Sherratt and Harvey, 1989), inhabiting pond bottoms and swimming through the water column to attack; and burrowers

115 (e.g., Golden-ringed dragonfly, *Cordulegaster boltonii*), burrowing in mud or bottom debris
116 and often lying in wait for prey with only the eyes protruding. The reliance on vision for
117 foraging varies among these types. For example, climbers that often have large compound
118 eyes rely extensively on vision for prey detection, while other types with smaller eyes may
119 rely more on tactile stimuli (Pritchard, 1966; Corbet, 1999). For tadpoles, the frequency of
120 encounters with and relative predation risk of these predators differ among habitats and
121 seasons. These differential predation risks likely determine the cost–benefit balance of
122 induced traits, thereby suggesting that tadpoles may exhibit different defense responses.
123 However, prior experimental studies have primarily focused on a single dragonfly species and
124 have not examined how tadpole responses may vary among odonate (and other) species.
125 The Japanese tree frog, *Dryophytes japonicus*, is a prevalent species with a wide
126 distribution encompassing entire regions of Japan (Honshu, Hokkaido, Shikoku, and Kyushu
127 islands), as well as regions outside Japan including Sakhalin Island, Jeju Island, and the
128 Korean Peninsula (Matsui and Maeda, 2018). Its breeding typically occurs from May to July
129 (in Honshu), with breeding pairs depositing small clutches of eggs at multiple sites. Tadpoles
130 of this species inhabit various environments such as rice paddies, marshes, ponds, puddles,
131 and other ephemeral water bodies (Matsui and Maeda, 2018). They are classified as
132 "nektonic" raspers, moving around the mid-water layer, characterized by distinctive body
133 shapes with tall tail fins that originate relatively anterior to the body (Orton, 1953). Tadpoles
134 of Japanese tree frog serve as important prey for numerous aquatic predators, including
135 dragonfly nymphs, water scorpions, and red-bellied newts (Ohba and Nakatsuji, 2006; Hirai
136 et al., 2020; Kita et al. 2020; Ozaki and Nishikawa, 2023). Morphological responses to
137 predators have also been documented in this species, with dragonfly nymphs inducing deep
138 tail fins, while chemical cues from goldfish prompt the development of shallow fins
139 (Takahara et al., 2012b). The occurrence of orange tail coloration has also been briefly
140 reported, although it has been attributed to ingested algae rather than predator-induced
141 plasticity (Doi et al., 2005; Yuasa, 2006, 2007). Despite being rarely documented, tadpoles

exhibiting brightly colored tails were frequently observed in our study site in Kyoto, central Japan (Noda, personal observation).

To clarify the cause and function of the orange tail coloration in tadpoles of *D. japonicus*, we conducted laboratory experiments with the tadpoles and several predator species. These experiments aimed to achieve two primary objectives. First, we investigated whether the tail coloration of tadpoles is induced by dragonfly nymphs, and if so, whether the intensity and speed of this response vary among different odonate species with different ecological characteristics. Simultaneously, we examined changes in body shape that are known to be plastically induced by predators. The second objective was to assess whether tadpoles exhibit color/morphological responses to predators characterized by a lower reliance on vision for foraging compared to odonates. We used water bugs (*Appasus major*), water scorpions (*Laccotrephes japonensis*), and newts (*Cynops pyrrhogaster*) as non-odonate predators. For such predators, predation avoidance strategies involving tail coloration are expected to be less effective than those against dragonfly nymphs. Thus, tadpoles may exhibit weakened or no color/morphological responses to minimize the costs associated with developing plastic traits. In both experiments, tadpoles were reared with a single predator species, and their morphological traits of interest were compared with those of tadpoles raised in predator-free tanks. By analyzing the extent of tail coloration in tadpoles exposed to diverse predators, we revealed that tadpoles modulate their induced responses according to the risks posed by predators with different foraging types and sensory capabilities.

Materials and Methods

Two series of laboratory experiments were carried out: Experiment I involved the comparison of induction of color/morphological changes in tadpoles by four different dragonfly species as predators, and Experiment II employed three non-odonate predators.

Experiment I

Adults of *Dryophytes japonicus* were captured in the paddy fields at Kyoto University (35°01'N, 135°47'E) on June 9 and 10, 2022. Following capture, mating pairs were housed overnight in plastic cages (19 × 19 × 14 cm; 6 cm water depth), and eggs were obtained the following day. After hatching, tadpoles were separately kept in plastic containers (43 × 31 × 16 cm; 6 cm water depth), as those from the same clutch were kept together in a single container. A room temperature of 20°C was maintained until the experiments began, and tadpoles were fed powdered food (Kyorin Co., Ltd.) every two days. In this experiment, four species of dragonfly nymphs were used as predators taking into account their foraging styles: *Anax nigrofasciatus* (Black-striped lesser emperor; climber), *Gynacantha japonica* (Blue-spotted dusk-hawker; climber), *Orthetrum albistylum speciosum* (White-tailed skimmer; burrower), and *Pantala flavescens* (Wandering glider; sprawler). Nymphs of *A. nigrofasciatus* collected in Sakura City, Chiba Prefecture, and those of *G. japonica* collected in Higashihiroshima City, Hiroshima Prefecture, were obtained from an online vendor, and *O. a. speciosum* and *P. flavescens* were collected from the same paddy field as the mating frog pairs. The dragonfly nymphs were maintained individually in plastic trays (23 × 17 × 8 cm) and fed tadpoles of *D. japonicus* every two days until the beginning of the induction experiment.

The induction experiment commenced on July 12, 2022. Each experimental tank housed 21 tadpoles, with three individuals randomly selected from each of the seven egg clutches to randomize genetic effects in each tank. Tadpoles in a tank were assigned to one of five treatments (Ax group with *A. nigrofasciatus*; Gy group with *G. japonica*; Or group with *O. a. speciosum*; Pf group with *P. flavescens*; and the control Ct1 group) with eight replicates per treatment. Each experimental tank (31 × 18 × 24 cm; 10 cm water depth) were equipped with a transparent isolation case (17 × 9 × 10 cm) with approximately 5 mm diameter holes spaced about 5 mm apart, where one dragonfly nymph was introduced. This setup allowed visual and chemical-mediated interaction between the predator and tadpoles without physical

contact. The tanks were aerated and maintained under a 14-h light/10-h dark photoperiod at a room temperature of 22°C, with half of the water in all tanks changed every three days. Air was continuously supplied to the isolation case within each tank through an aquarium air stone. Tanks designated for the Ct1 group lacked predators and utilized an empty isolation case. Tadpoles in the experimental tanks were fed every two days with commercial food for Axolotl, *Ambystoma mexicanum* (Kyorin Co., Ltd.), while dragonfly nymphs received two tadpoles as food every two days.

Experiment II

The design of Experiment II mirrored that of Experiment I, with adjustments made for sampling dates, the interval between hatching and the experiment's commencement, and the selection of predators. For this experiment, adults of *D. japonicus* were captured from the paddy fields at Kyoto University on May 18 and 20, 2022. Eggs were collected and tadpoles from clutches were reared in the same way as in Experiment I until the experiments began. Three predator species were chosen based on their foraging style and the primary sensory organs used for prey detection: Ferocious water bug, *Appasus major* (sit-and-wait predator using tactile and olfactory senses), water scorpion, *Laccotrephes japonensis* (sit-and-wait predator using tactile and olfactory senses), and Japanese fire belly newt, *Cynops pyrrhogaste*r (active tracker using olfactory senses). Aquatic insects, collected in Higashihiroshima City, were purchased from the online vendor, while red-bellied newts were collected from irrigation ditches in Kyoto City (35°05'N 135°47'E). These predators were individually housed in plastic trays and fed with tadpoles every two days.

The induction experiment began on June 18, 2022. Each experimental tank housed 21 tadpoles, with seven individuals randomly selected from each of the three egg clutches. Tadpoles in each tank were allocated to one of four treatments (Be group with *A. major*; Ws group with *L. japonensis*; Fn group with *C. pyrrhogaster*; and the control Ct2 group) with eight replicates per treatment. One predator was introduced into an isolation case in each tank.

Other experimental setups, i.e., tank design, room temperature, light condition, and feeding frequency, were kept the same as in Experiment I.

Measuring Coloration and Morphological Responses

All tadpoles were photographed two and three weeks after the start of the experiment using a Canon® EOS RP mirrorless camera. Individual tadpoles were placed in a narrow acrylic aquarium with a ruler and color chart in the background. The distance between the aquarium and the camera was kept constant for all individuals. All photos were taken with a F/16 aperture, a 1/20th second exposure time, and ISO 3200. Consistency in artificial lighting conditions was maintained for individuals photographed on the same day. Some individuals were excluded from the analysis due to poor nutritional condition or significant tail loss during rearing. The exclusion criteria included: (1) head and body length less than 6 mm, (2) severe tail damage or loss, and (3) inability to maintain posture in water.

Tail parts excluding the tail muscle were trimmed using the image measuring software, Natsumushi version 1.12.2 (Tanahashi and Fukatsu, 2018; https://sites.google.com/site/mtahashilucanid/program/natsumushi). The color of the tail was quantified using the average of CIELAB values (CIE 1976) calculated in Adobe Photoshop 25.5.1 (Adobe Inc.). In the CIELAB color space, color was represented using three components: the lightness component L* and the color components a* and b*. The L* component ranged from black to white between 0–100, while a* and b* components ranged from green to red and blue to yellow, respectively, on a scale of −128 to 127. As Photoshop displays images in 8-bit format, these values were rescaled to range from 0 to 255. Therefore, L* was multiplied by 100/255 and a* and b* were adjusted by subtracting 128, respectively, to facilitate analysis (Fig. 1). Due to an incorrect white balance, photographs taken at the second week of Experiment II were excluded from the analysis of tail coloration. During the third week of Experiment II, photographs of 11 individuals from the Ct2 group were also taken with an incorrect white balance, so the color tone was later corrected using Photoshop,

referencing one of the other photographs from the Ct2 group. Additionally, morphological measurements were conducted based on the photographs using Natsumushi. Morphological traits included tadpole body length (BL), tail length (TAL), and tail depth (TD) were measured using the scale within each image.

Statistical Analyses

All statistical analyses were conducted in R version 4.3.2 (R Core Team, 2023). Linear mixed models (LMMs) were employed based on the measured traits, using the lmerTest package (Kuznetsova et al., 2017) to estimate differences between the control group and the other treatments. The experimental tank was treated as a random factor in the LMMs. When color components (e.g., a* and b*) were utilized as the response variables, the treatment (predator) was used as the explanatory variable in the models to compare the treatment groups to the control group. In the same way, when body length (BL) was utilized as the response variables, the treatment was used as the explanatory variable in the models. On the other hand, in analyses for TAL and TD as the response variable, four models were constructed for each trait. These models included: the model with only BL as the explanatory variable, the model with only the treatment as the explanatory variable, the model with both BL and treatment as explanatory variables, and the model incorporating BL, treatment, and their interactions. The final model was determined via backward elimination, by applying likelihood ratio (LR) tests. Specifically, all explanatory variables (with their interactions) were entered into the full model, then removed one by one until a final model was selected. At each step, the reduced model was compared with the former model by LR test. The significance level was set at 5%. If a significant difference was found between the former and reduced models, the former model was adopted as the final model for further analysis.

After constructing final models, comparisons between each treatment group and the control were performed using Dunnett's test with the glht function in the multcomp package (Hothorn et al., 2008). The significance level was set at 5%, with treatment groups considered

277 significantly different from the control group if the *P* values were below this threshold.
278 Orange tail coloration and increased tail depth were reported as predator-induced traits in
279 previous studies, so we decided to focus primarily on b* values and tail depth.
280 We also conducted a principal component analysis (PCA) for color and
281 morphological traits at the third week after the start of the experiment to concisely visualize
282 the multivariate responses of the tadpoles to the predator treatments. The analysis included
283 total length (TL; the sum of BL and TAL), standardized body length (sBL), standardized tail
284 length (sTAL), standardized tail depth (sTD), and coloration values (a* and b*), The sBL,
285 sTAL, and sTD were calculated by dividing the BL, TAL, and TD measurements by TL, then
286 subtracting their respective means and dividing by their standard deviations, in order to use
287 variables with different units together. Pearson's correlation matrices were used to compare
288 the principal component scores with the original variables to interpret the principal
289 components. We also used Pearson's correlation matrices to assess the relationships among
290 tadpole traits.
291
292
293 **Results**
294 **Coloration**
295 In Experiment I, the values of a* were lower in the Ax group compared to the Ct1
296 group (Dunnett's test, $P < 0.001$; Fig. 2). Additionally, the Ax group had a higher value of b*
297 ($P < 0.001$). The latter indicates that tadpoles in the Ax group showed more orange tails than
298 in the control group. Also at the third week, the values of a* were lower ($P = 0.0283$) and b*
299 were higher ($P < 0.001$) in the Ax group than in the control group. In addition, the Or group
300 showed higher b* values than the Ct1 group ($P = 0.0443$), indicating that orange tails were
301 induced not only by *Anax nigrofasciatus*, but also by *Orthetrum albistylum speciosum*. Some
302 individuals of the Pf group also exhibited tail coloration, but this tendency was not

statistically significant ($P = 0.1426$ in b* value). However, in Experiment II, no group showed significant differences from the Ct2 group in the a* or b* values at the third week (Fig. 3).

Morphological Responses

In Experiment I, whether tail length or height was used as the response variable, models with BL and treatment as explanatory variables were selected as the final models at both the second and third weeks after the start of the experiment. At the second week, the Pf group had a larger body compared to the Ct1 group (Dunnett's test, $P = 0.0471$; Fig. 4), and tails were shorter in the Ax and Pf groups than in the Ct1 group ($P < 0.001$). Moreover, the Ax group had a higher tail than the Ct1 group ($P = 0.0092$). At the third week, only the Gy group had a shorter tail than the Ct1 group ($P = 0.047$), while the Pf group, as well as the Ax group, showed a higher tail than the Ct1 group ($P = 0.0215$ for Pf, $P < 0.001$ for Ax). The Or group also tended to have higher tails compared to the Ct1 group, but the difference was not statistically significant ($P = 0.1132$).

In Experiment II, the models with BL and treatment as explanatory variables were chosen in the model selection for tail length at the second week. On the other hand, at the third week, the models with only BL were selected. As for tail depth, the models with only BL were also selected. Although the models with only BL as explanatory variable indicated that the effect of the treatment on tail length or tail depth was not large, post-hoc tests were conducted to assess differences between treatments. At the second week, the Be group exhibited a shorter tail ($P < 0.001$), but no significant differences in tail depth were observed between any group and the Ct2 group ($P > 0.05$ for all Be, Ws, and Fn; Fig. 5). At the third week, there was no significant difference in tail depth compared to the Ct2 ($P > 0.05$ for all Be, Ws, and Fn). Consequently, tadpoles in all non-odonate predator treatments were not induced to develop significantly deeper tail fins compared to the control group.

Principal Component Analysis

In Experiment I, the first two principal components (PC1 and PC2) for larval coloration and morphology accounted for 67.3% of the variation, then combined with PC3, they explained 83% (Table 1). PC1 showed strong correlations with all morphological traits except sTD, indicating that it is likely influenced by the larval body proportion between body length and tail length. On the other hand, PC2 was strongly correlated with sTD (Pearson's correlation coefficients, -0.84) and b* (-0.65), suggesting that it represented the direction of predator-induced responses of *Dryophytes japonicus*. The 95% probability ellipse of the Ax group was conspicuously elongated in the negative direction along the PC2 axis, followed by the Pf, Or and Gy groups (Fig. 6). An increase in tail height and coloration change occurred together; the correlation coefficient between sTD and b* value was 0.34, ranking as the fourth highest after the coefficients between TL and other morphological traits (sBL, sTAL), and between TL and b* value (Table 2).

In Experiment II, the first two principal components explained 77.5% of the variation, and combined with PC3, they accounted for 90.4% (Table 1). PC1 largely explained color trait variation, as well as morphological traits except sTD. PC2 exhibited strong correlations with sBL, sTAL, and sTD, while PC3 showed relatively strong correlations with sTD and b*. Although PC3 may represent the direction of response in this species, its overall contribution to the total variation was limited (12.9%). Furthermore, the correlation between sTD and b* value was 0.16, which was not particularly large compared to other coefficients (Table 2), indicating that changes in tail color and shape did not necessarily occur together.

Discussion

Tail Coloration in Asian Species of *Dryophytes*

Our experiments revealed that tadpoles of *Dryophytes japonicus* developed induced changes in tail coloration as well as tail shape in response to dragonfly nymphs. Although tail coloration has rarely been documented in this species, its occurrence has been reported from

357 several locations in Japan, including Hokkaido, Shiga, Hyogo, Hiroshima, Shimane, Kouchi
358 and Ehime prefectures (Doi et al., 2005), as well as from our collection site in Kyoto (Noda,
359 personal observation). The phenotypes induced by *Anax nigrofasciatus*, i.e., deep tail fins and
360 bright orange tails, closely resemble those previously observed in other species in *Dryophytes*
361 in the New World, Cope's Gray Treefrog (*Dryophytes chrysoscelis*; McCollum and Van
362 Buskirk, 1996), Pine Woods Treefrog (*Dryophytes femoralis*; LaFiandra and Babbitt, 2004),
363 and Gray Treefrog (*Dryophytes versicolor*; Van Buskirk and McCollum, 1999). Thus, our
364 findings represent the first documented case of a predator-induced tail coloration phenotype in
365 Asian species of *Dryophytes*.

366 Our discoveries of the plasticity in the tail coloration in the Asian species of
367 *Dryophytes* suggests that the trait is symplesiomorphically shared between the North
368 American and ancestral Asian lineages, and has been inherited by present-day Asian species,
369 including *D. japonicus*. An ancestral stock of *Dryophytes* is inferred to have migrated from
370 North America to East Asia across the Bering Land Bridge during the Miocene (Duellman et
371 al. 2016; Dufresnes et al., 2016). Present-day Asian species of *Dryophytes* are monophyletic,
372 having diversified from this ancestral stock. It is plausible that tail coloration has persisted as
373 a phenotypic plasticity in both North American and East Asian species of *Dryophytes* due to
374 the consistent importance of dragonfly nymphs as predators.

375 It remains unknown whether tail coloration can be induced in the three other Asian
376 species, *Dryophytes flaviventris*, *Dryophytes immaculates*, and *Dryophytes suweonensis* (see
377 Borzée et al., 2020). In North America, Pine Barrens Treefrog, *Dryophytes andersonii* is
378 induced to have a darker tail, not an orange tail, by *Anax junius*, which induces orange tails in
379 other species of *Dryophytes* (Kruger and Morin, 2020). Furthermore, Squirrel Treefrog,
380 *Dryophytes squirellus* exhibits no morphological changes in response to odonate species
381 (McCoy and Bolker 2008; Baker, 2021). These cases indicate that the plastic responses in
382 *Dryophytes* have diversified, and some may have lost the ability to develop orange tails.

383 Future studies are needed to elucidate the interspecific variation in plastic responses to
384 predation risk and the evolutionary causes underlying this variation.
385
386 **Differences in the Extent of Coloration by Predators**
387 Our experiments demonstrated that the extent of induced responses varied depending
388 on the dragonfly species reared with tadpoles. Two weeks after the start of the experiment,
389 nymphs of *A. nigrofasciatus* induced both deep fin and tail coloration in tadpoles, whereas, at
390 the third week, *Pantala flavescens* also induced deep tail and *Orthetrum albistylum speciosum*
391 induced tail coloration. Furthermore, although weak, some tadpole individuals exposed to *P.*
392 *flavescens* showed tail coloration, and those exposed to *O. albistylum speciosum* also tended
393 to have higher tails. These results indicate that the defense traits (coloration and deep tail)
394 were induced by the nymphs of *A. nigrofasciatus* more rapidly and strongly, but similar
395 responses were also elicited by the other two predator species. The probability ellipses in PCA
396 further suggest that the direction of larval responses to these benthic predators (*P. flavescens*
397 and *O. albistylum speciosum*) were similar to that to *A. nigrofasciatus* (Fig. 6).
398 The differences in the degree and rate of responses induced by different dragonfly
399 species may reflect varying levels of predation risk for tadpoles posed by each species. Plastic
400 phenotypes with significant tail coloration should result in trade-offs because of energetic
401 costs associated with synthesizing pigmentation (Grether et al., 2001) or costs due to the
402 increased vulnerability to mismatched predators. Consequently, tadpoles are expected to
403 minimize these costs by developing effective defense traits for each predator as much as
404 possible based on the perceived predation risk (Touchon and Warkentin, 2008; Innes-Gold et
405 al., 2019). The predation risk for tadpoles would be different among dragonfly species
406 according to (1) the foraging style of dragonfly nymphs, (2) their color vision, and (3) the
407 total amount of intake related to their body size and occurrence period.
408 Firstly, the four dragonfly species used in our experiment exhibit different foraging
409 styles. According to the classification by Corbet (1999), *A. nigrofasciatus* and *G. japonica* are

410 climbers, sit-and-wait predators on aquatic vegetation, while *P. flavescens* is a sprawler,
411 foraging while moving along the bottom, and *O. albistylum speciosum* is a burrower, residing
412 within the substrate and ambushing prey. For sit-and-wait predators, there is often a time lag
413 between identifying prey and launching an attack, during which they can choose which part of
414 tadpole's body to target. Therefore, the lure effect of tadpole tails is likely to attract ambush
415 predators more effectively than those that actively chase their prey. In addition, as *A.*
416 *nigrofasciatus* prefers habitats abundant in floating-leaved plants (Ozono et al., 2021), it can
417 move freely along the plant structures from roots anchored at the bottom to leaves extending
418 above the water surface. As a result, the risk of predation on tadpoles could be present
419 throughout the entire water column, from the bottom to the surface, making behavioral
420 responses like moving to a specific depth insufficient as a means of avoiding predation.
421 Conversely, *P. flavescens* and *O. albistylum speciosum* primarily forage at the bottom, so
422 tadpoles may evade these predators by relocating to higher water layers. However, such
423 avoidance behavior often leads to a decrease in growth rate due to reduced foraging efficiency
424 (Teplitsky et al., 2003); i.e., for tadpoles that primarily feed on detritus, avoiding the bottom
425 layer may result in malnutrition. The combination of moderate morphological responses with
426 behavioral responses (the "double defense" strategy; Dewitt and Hucko, 1999) may help
427 mitigate the impact of malnutrition from behavioral changes while minimizing the costs of
428 defensive traits.

429 Secondly, differences in eyesight, particularly color vision among dragonfly species,
430 may influence the extent of the tail coloration changes in the tadpoles. Pritchard (1966) noted
431 a correlation between foraging habit and compound eye size in dragonfly nymphs, with
432 climbers typically possessing larger compound eyes compared to sprawlers and burrowers. He
433 also stated that the climbers, such as species in *Anax*, rely heavily on vision for prey
434 detection, whereas other foraging types depend more on tactile stimuli. Hence, it is plausible
435 that the lure effect of the brightly colored tails is more effective for climbers, which are more
436 visually oriented. Furthermore, Futahashi et al. (2015) investigated the genetic basis of color

437 vision in dragonflies and redocumented the number of opsin genes and the characteristic
438 spatiotemporal expression patterns in the nymphs of various species, including those of the
439 burrower *O. albistylim* and the climber *A. parthenope*, a species closely related to *A.*
440 *nigrofasciatus* used in our experiment. They found that dragonflies possess a greater number
441 of opsin genes than other insects, and *A. parthenope* possesses and expresses a greater number
442 of opsin genes, particularly those sensitive to long-wavelength light. Although the functions
443 of these diverse genes are not fully understood, this suggest that species of *Anax* may have
444 enhanced abilities to perceive reddish colors, potentially enabling tadpoles with bright orange
445 tails to more effectively avoid predation by using their tails as lures. Thus, the larval
446 antipredator defense of *D. japonicus*, which involves tail coloration, may have been driven by
447 the sensory systems of predatory dragonfly nymphs, and subsequently evolved specifically
448 against color-sensitive predators, represented by dragonfly nymphs of *Anax*.
449 Thirdly, the variation in the degree of induced responses may reflect the relative
450 predation risks associated with body sizes or occurrence periods of respective odonate
451 predators. In nature, it is generally difficult to determine which predators exert higher
452 predation pressure on tadpoles, and few studies have investigated such differences. However,
453 during the rearing process, we observed that the nymphs of *A. nigrofasciatus* exhibited higher
454 food demands and displayed more frequent attacks on tadpoles compared to other species.
455 Additionally, they are approximately twice the size of the nymphs of *P. flavescens* and *O.*
456 *albistylum speciosum* (see Ishida et al., 1988), suggesting that size-related predation pressures
457 could influence the necessity and effectiveness of tadpole morphological responses. However,
458 tadpoles did not exhibit significant responses to *G. japonica*, which is similar in size to *A.*
459 *nigrofasciatus* (see Ishida et al., 1988). This indicates that tadpoles likely respond not only to
460 size-related predation pressure but also to other characteristics of dragonfly species, such as
461 foraging style or habitat overlap, thereby modulating the extent of induced responses. Since
462 the habitat of nymphal *G. japonica* overlaps with that of tadpoles of *D. japonicus* (Nakagawa
463 et al., 2004), the lack of an apparent induced response may be due to the limited period in

464 which they co-occur. This species grows more rapidly compared to other species in Aeshnidae
465 in Japan, occurring in aquatic environments only from May to August (Ozono et al., 2021).
466 Considering that the nearly-metamorphosing nymphs cease foraging, the period during which
467 this species poses a predation threat to *D. japonicus* is even shorter. This brief coexistence
468 period suggests that this dragonfly species may not be an important predator for the tadpoles.
469 In addition to the tail coloration and depth we focused on, a tendency toward larger
470 body lengths and shorter tail lengths compared to those in the control group was observed,
471 although not statistically significant in most treatment groups. These changes may represent
472 general responses to predator treatments, as noted in previous studies. The increase in body
473 length could be attributed to enhanced availability of food resources resulting from leftovers
474 and feces of caged predators (e.g., McIntyre et al., 2004; Richardson, 2006). Similarly, shorter
475 tail lengths in the presence of predators have been reported in several frog species (Laurila et
476 al., 2002; LaFiandra and Babbitt, 2004).
477
478 **Responses Toward Non-Odonate Predators**
479 Tadpoles of *D. japonicus* showed neither conspicuous tail coloration nor deep fin
480 when exposed to newts or aquatic insects other than dragonfly nymphs in our experiment,
481 likely because the costs of expressing these defenses outweighed their benefits for avoiding
482 predation. Although Experiment II did not include a positive control (i.e., treatment with
483 dragonfly nymphs, such as *A. nigrofasciatus*), we believe that the absence of color induction
484 was unlikely caused by experimental failure, because preliminary experiments demonstrated
485 the high reproducibility of the induced responses when using dragonfly nymphs. Therefore,
486 our results suggest that changes in body color and tail depth are not strongly induced by these
487 predators and may not serve as effective predation avoidance strategies against them. An
488 important point in understanding why tadpoles did not show tail coloration is that defensive
489 traits come with physiological costs and may increase predation pressure from non-focal
490 predators. If changes in tail color and shape do not significantly contribute to non-odonate

491 predator avoidance, tadpole should minimize these disadvantages by refraining from
492 developing these antipredator defenses.

493 Given that the non-odonate predators are presumably less dependent on vision than
494 odonate species when foraging, the strategy of using colored deep tails as lures may be
495 ineffective and provides only limited benefits against them. For instance, diving beetles, water
496 bugs, and water scorpions capture prey by grasping the entire body with their arms, so the tail
497 of tadpoles should not serve as a lure for them. Additionally, they use tactile, visual, and
498 olfactory senses for locating prey (Babbitt and Jordan, 1996; Kehr and Gómez, 2009; Mogali
499 et al., 2019; Nowińska and Brożek, 2019), and possess well-developed organs for detecting
500 auditory and mechanical signals (Yager, 1999; Cocroft and Rodríguez, 2005), suggesting they
501 are relatively less dependent on vision. Previous studies have reported predator-induced
502 changes in larval morphology in response to these predators (Benard, 2006; McCoy, 2007;
503 Michel, 2012), but few have reported effects on larval tail coloration (Touchon and
504 Warkentin, 2011). Similarly, newts and salamanders, both as larvae and adults, are active
505 foragers that swallow their prey whole like fish (Wilson et al., 2005; Kishida and Nishimura,
506 2007). Consequently, the tails of the tadpoles are unlikely to function as a lure for these
507 predators. Newts tend to rely on olfaction cues as well as vision for food recognition
508 (Copeland, 1913; Lindquist and Bachmann, 1982; Schwarz et al., 2020). Few studies have
509 reported that tadpoles exhibit induced defensive traits in response to these predators, and none
510 have documented induced changes in body coloration (Kishida and Nishimura, 2004; Hettyey
511 et al., 2011; Urban et al., 2017). These facts support the hypothesis that the brightly colored
512 tails of *D. japonicus* do not function as lures against the non-odonate predators, and that, as a
513 result, tadpoles do not express costly defensive traits in response to them.

514 The lack of induction of defensive phenotypes may also be attributed to
515 physiological costs associated with developing tail coloration, although the magnitude of such
516 costs in tadpoles remains poorly understood. Since most animals are unable to biosynthesize
517 carotenoids, the pigments thought to be causing the bright orange coloration, they metabolize

and accumulate these pigments by absorbing them from their diet (Bertrand et al., 2006; Thibaudeau and Altig, 2012). Thus, the allocation costs of expressing coloration by transporting these pigments may not be as high as commonly assumed. On the other hand, the potential for increased vulnerability to non-focal predators may also interfere with the expression of defensive traits (Innes-Gold et al., 2019). This disadvantage may sometimes outweigh the energetic costs of expressing a defensive phenotype. Carfagno et al. (2011) found that tadpoles of *Acris* exhibit darker a tail spot, which is considered a defensive trait against dragonfly nymphs, even in the absence of predators. However, the presence of fish predators induces the loss of this spot. This suggests that the cost of expressing defensive traits may be lower than typically expected, and the disadvantage of increased vulnerability to mismatched predators may prevent these phenotypes from being constitutively expressed. Similarly, in tadpoles of *D. japonicus*, the elevated risk of predation from non-odonate predators, considered non-focal predators, due to expressing bright tail coloration, rather than its energetic cost, could explain why they did not exhibit deep and brightly-colored tail in response to these predators. These defense traits against dragonfly nymphs may not only be ineffective in preventing predation by non-odonate predators but could also increase the potential vulnerability to them. For example, the deepened tail may increase the vibrations produced during swimming, thereby heightening predation pressure on tadpoles by tactile or olfactory predators (i.e., water bugs, water scorpions, and newts). Additionally, for other predators such as fish, tail coloration may make the tadpoles more vulnerable by increasing their visibility. However, further experiments are warranted to assess and compare the effectiveness of these defense traits toward each predator, including evaluating how tadpole coloration influence their survival rate in the presence of various aquatic predators.

Acknowledgements

We thank the Experimental Farm, Graduate School of Agriculture, Kyoto University, for providing the research environment, and Mr. Kagata, Mr. Yasui, and Mr. Sakakibara, technical staff, for their assistance with observations. This work was supported by JST SPRING, Grant Number JPMJSP2110.

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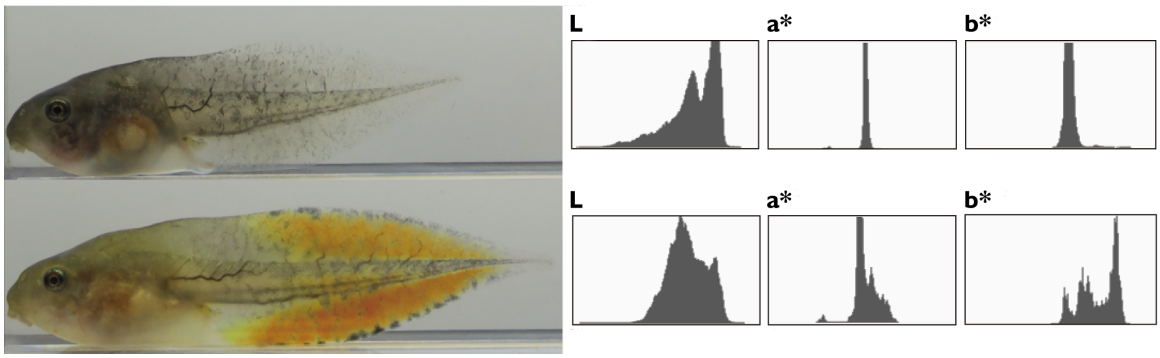

Fig. 1. Photographs of tadpoles of *Dryophytes japonicus* reared without predator (top) and with *Anax nigrofasciatus* (bottom) and their corresponding Photoshop Lab color histograms. The mean values of tail coloration are L = 61.2, a* = -2.5, and b* = 7.96 (without predator), and L = 57.96, a* = 1.75, and b* = 35.51 (with *A. nigrofasciatus*), respectively.

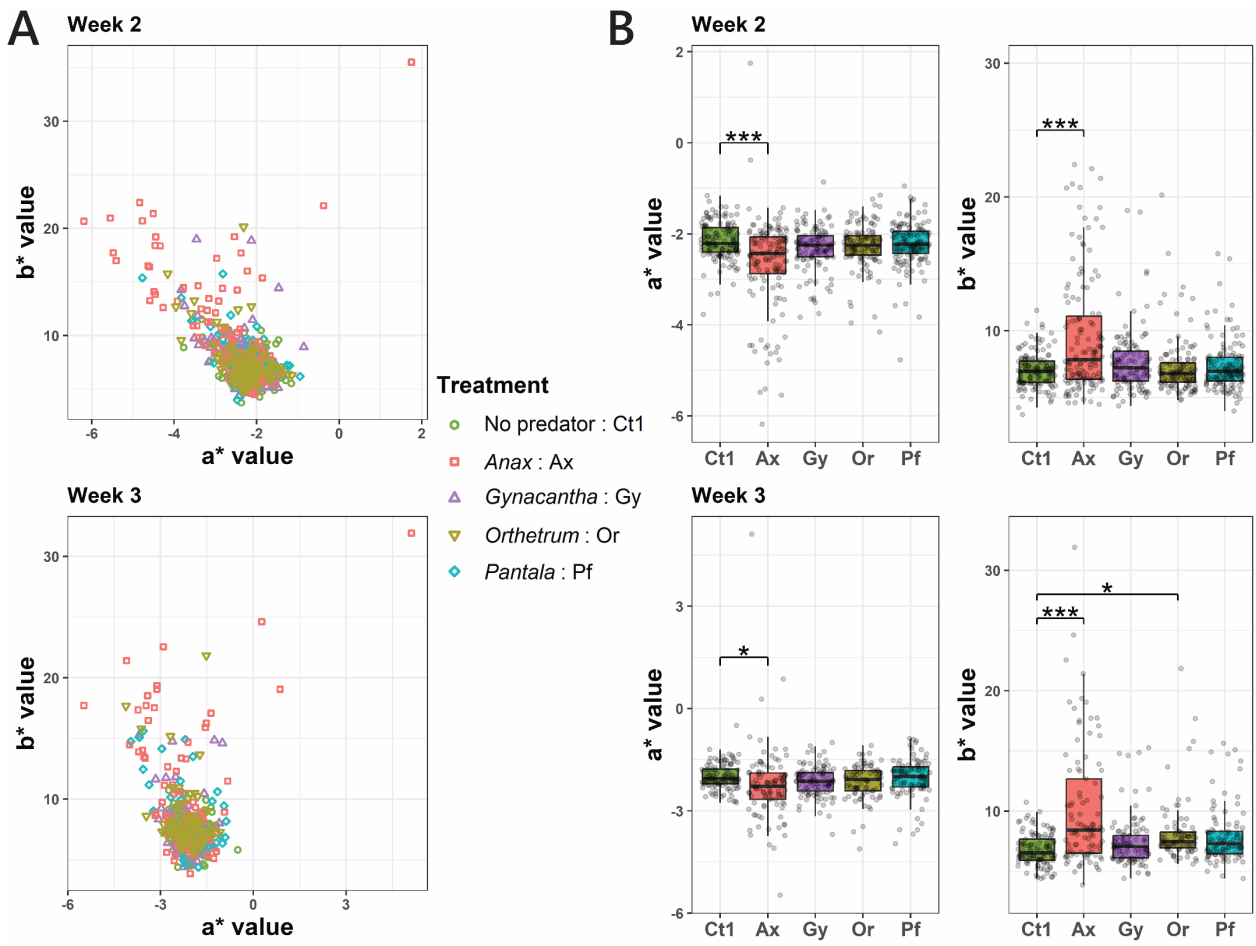

Fig. 2. (A) Scatterplots of tail coloration parameters (a* and b* values) of tadpoles two weeks (top) and three weeks (bottom) after the start of the Experiment I, using different dragonfly species. Tadpoles were reared in the presence of no predators (the control group; Ct1), *Anax nigrofasciatus* (Ax), *Gynacantha japonica* (Gy), *Orthetrum albistylum speciosum* (Or), or *Pantala flavescens* (Pf). (B) Box-and-whisker plots displaying the range of the tail color traits (a* and b* values) two weeks (top) and three weeks (bottom) after the start of the Experiment I. The asterisks in the figure indicate that there were significant differences compared to the control group, which were led by the LMM analysis (see Materials and Methods). *$P < 0.05$, ***$P < 0.001$.

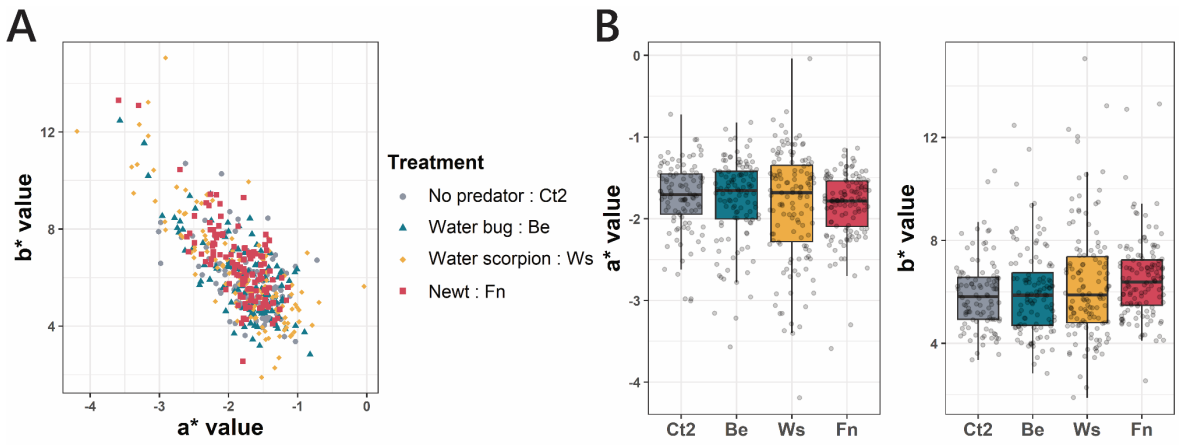

Fig. 3. (A) Scatterplots of tail coloration parameters (a* and b* values) of tadpoles two weeks (top) and three weeks (bottom) after the start of the Experiment II, using non-odonate species. Tadpoles were reared in the presence of no predators (the control group; Ct2), water bug (*Appasus major*; Be), water scorpion (*Laccotrephes japonensis*; Ws), and fire belly newt (*Cynops pyrrhogaster*; Fn). (B) Box-and-whisker plots displaying the range of the tail color traits (a* and b* values) three weeks after the start of the Experiment II.

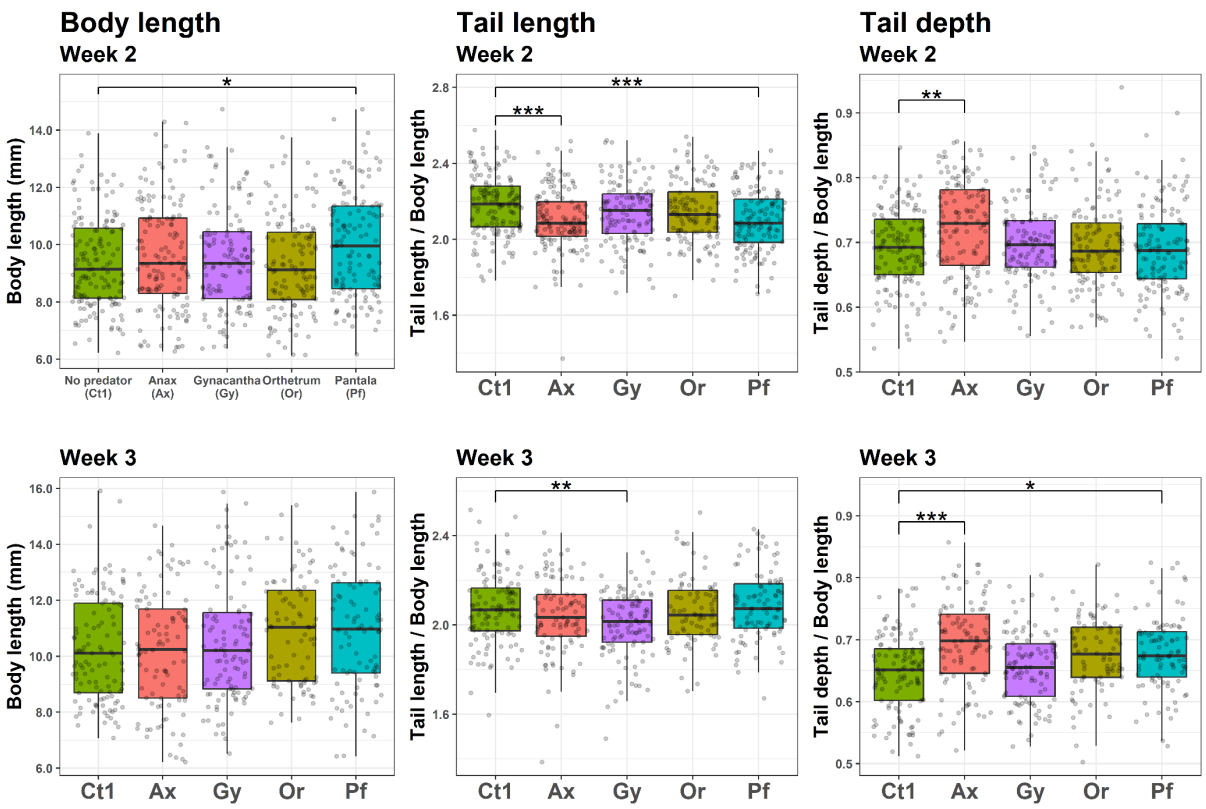

Fig. 4. Box-and-whisker plots showing the range of tadpole morphological traits, BL (body length), TAL (tail length), and TD (tail depth) in Experiment I, using different dragonfly species. Tadpoles were reared in the presence of no predators (the control group; Ct1), *Anax nigrofasciatus* (Ax), *Gynacantha japonica* (Gy), *Orthetrum albistylum speciosum* (Or), or *Pantala flavescens* (Pf). TAL and TD values were divided by body length to account for size differences among individual tadpoles. The asterisks in the figure indicate that there were significant differences compared to the control group, which were led by the LMM analysis (see Materials and Methods). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

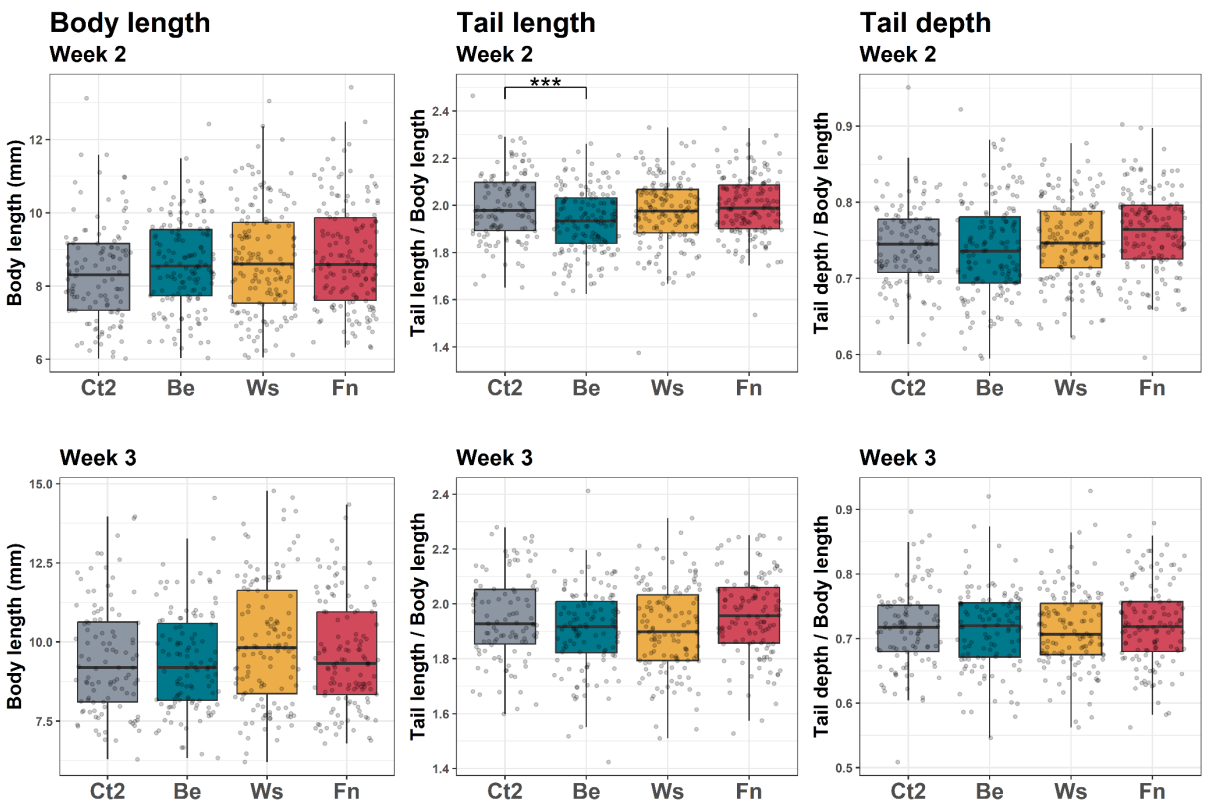

Fig. 5. Box-and-whisker plots showing the range of tadpole morphological traits, BL (body length), TAL (tail length), and TD (tail depth) in Experiment II, using non-odonate predators. Tadpoles were reared in the presence of no predators (the control group; Ct2), water bug (*Appasus major*; Be), water scorpion (*Laccotrephes japonensis*; Ws), and fire belly newt (*Cynops pyrrhogaster*; Fn). TAL and TD values were divided by body length to account for size differences among individual tadpoles. The asterisks in the figure indicate that there were significant differences compared to the control group, which were led by the LMM analysis (see Materials and Methods). ***P <0.001.

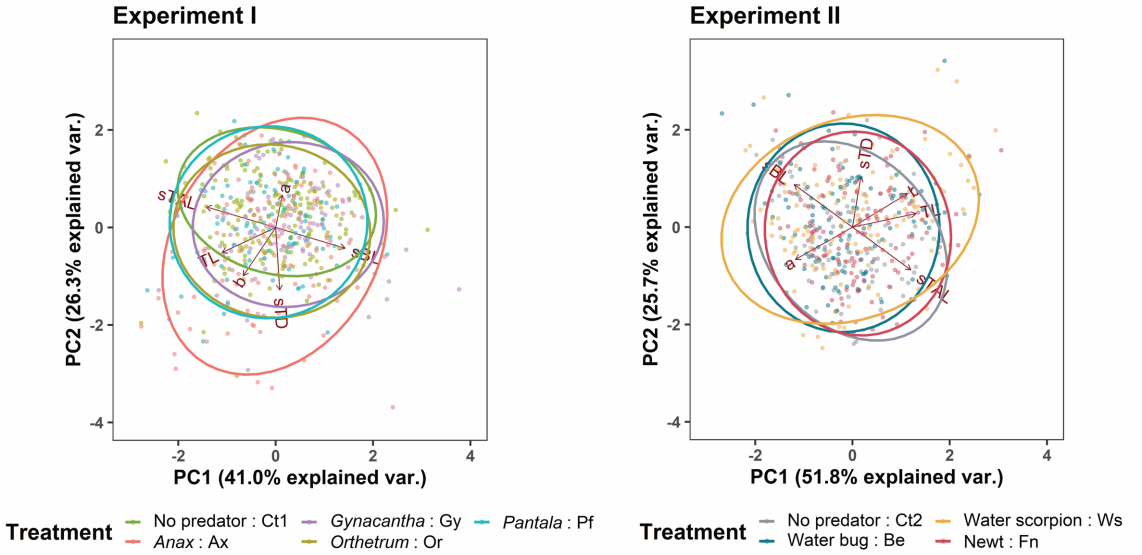

Fig. 6. Plots of principal components 1 and 2, with their contribution shown in parentheses, based on four morphological traits, total length [TL; the sum of body length (BL) and tail length (TAL)], standardized body length (sBL; BL/TL), standardized tail length (sTAL; TAL/TL), and standardized tail depth (sTD; TD/TL), and the coloration values (a* and b*), sampled three weeks after the start of Experiment I (left) and Experiment II (right). In Experiment I, tadpoles were reared in the presence of no predators (the control group; Ct1), *Anax nigrofasciatus* (Ax), *Gynacantha japonica* (Gy), *Orthetrum albistylum speciosum* (Or), or *Pantala flavescens* (Pf). In Experiment II, they were reared in the presence of no predators (the control group; Ct2), water bug (*Appasus major*; Be), water scorpion (*Laccotrephes japonensis*; Ws), and fire belly newt (*Cynops pyrrhogaster*; Fn). Ellipses represent the 95% probability ellipses of each treatment group. The red arrows indicate the direction in which the variables of each trait contribute to the principal components. Data for one individual in the Ax group, which appeared as an outlier in the bottom left-hand side, was omitted in this figure.

Table 1. Results of principal component analysis of coloration and morphological traits of tadpoles in Experiment I (top) and Experiment II (bottom). PC1, PC2, and PC3 indicate the first three principal components. The values in the table are the correlation coefficients of each morphological trait with the principal component scores.

Experiment I

Response variable	PC1	PC2	PC3
Total Length	–0.72	–0.34	0.19
Standardized body length	0.93	–0.28	0.10
Standardized tail length	–0.93	0.28	–0.10
Standardized tail depth	0.05	–0.84	0.07
a*	0.09	0.43	0.88
b*	–0.44	–0.65	0.31
% of variance explained	41.0%	26.3%	15.7%

Experiment II

Response variable	PC1	PC2	PC3
Total Length	0.85	0.18	0.06
Standardized body length	–0.78	0.57	0.22
Standardized tail length	0.78	–0.57	–0.22
Standardized tail depth	0.11	0.68	–0.72
a*	–0.77	–0.43	–0.15
b*	0.74	0.45	0.36
% of variance explained	51.8%	25.7%	12.9%

871 **Table 2.** The Pearson's correlation coefficients between morphological trait pairs, total
872 length [TL; the sum of body length (BL) and tail length (TAL)], standardized body length
873 (sBL; BL/TL), standardized tail length (sTAL; TAL/TL), and standardized tail depth (sTD;
874 TD/TL), and the coloration values (a* and b*). Diagonal cells, representing self-correlations,
875 are denoted by dashes. Results from Experiment I are shown above the diagonal, while results
876 from Experiment II are presented below the diagonal.

Experiment I

	TL	sBL	sTAL	sTD	a*	b*
TL	–	–0.47	0.47	0.18	–0.07	0.43
sBL	–0.51	–	–1.00	0.20	0.03	–0.18
sTAL	0.51	–1.00	–	–0.20	–0.03	0.18
sTD	0.16	0.14	–0.14	–	–0.21	0.34
a*	–0.62	0.33	–0.33	–0.26	–	–0.10
b*	0.67	–0.26	0.26	0.16	–0.72	–

Experiment II

877