

Predation promotes diversification in the mean and variance of antipredator traits

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Abstract

1. Predator species are separated along habitat gradients, with predation known to play an essential role in species' trait diversification. Because predator species differ dramatically in their hunting style and mode, a change in predator species will alter the mean and variance of prey's antipredator traits. Population trait variation has an impact on community ecology. It influences species' niche width and interactions in the food web. However, empirical studies on variance change by predation are scarce.
2. In this study, we collected large numbers of the last instar *Leucorrhinia pectoralis* exuviae from lakes with predatory fish (fish lakes) and lakes with large invertebrate predators (dragonfly lakes) and compared their two antipredator traits (spines and body size).
3. We found that individuals in dragonfly lakes grew shorter spines than individuals in fish lakes. Body size showed no significant difference between dragonfly-lake individuals and fish-lake individuals. Moreover, populations from dragonfly lakes showed a smaller variance of spine length than populations from fish lakes; while populations from dragonfly lakes had a larger variance in body size than populations from fish lakes.
4. These results indicate that trait variance, as well as mean, is strongly modified by different predation regimes. Studying the mean and variance of traits can help to define the mode of selection forces (directional selection and stabilising selection) in nature. Moreover, dragonfly larvae might be ideal organisms for the study of phenotypic selection on quantitative traits in the wild.

KEYWORDS

dragonfly larvae, mean and variance, phenotypic selection, population diversification, predation regimes shift

INTRODUCTION

Predators represent a major source of selection shaping prey phenotypes (Benard, 2004; Vamosi, 2005). As a response prey species have evolved a large array of traits to avoid and repel predators (Benard, 2004; Edmunds, 1974; Schmitz, 2017). Because predators

are restricted to different habitats, prey species often segregate along predator gradients (McPeck, 1990a; Mikolajewski et al., 2006; Stoks & McPeck, 2003; Swaegers et al., 2017). Key antipredator traits of prey are expected to influence the outcome of predator-prey interactions, and thus, these traits determine the occurrence of prey along predator gradients (McPeck, 1990a; Petrin et al., 2010). Body size, which is

directly linked to fitness (McArdle & Lawton, 1979), is a classical trait for studying predator selection on phenotypes because different predators select for either small prey size (Juanes, 1992; Pickering et al., 2017) or favour large prey (Black, 1993; Manca et al., 2008). Besides body size, other morphological defences against predators have been widely studied, because these defences often have prominent and striking features (Bourdeau & Johansson, 2012). Among the spines, a typical defensive trait has received much attention because they protect a large array of prey species against predators (Dahl & Peckarsky, 2002; Johansson & Mikolajewski, 2008; Murphy et al., 2010; Vamosi & Schluter, 2004).

Many previous studies have shown that predation can cause morphological divergence in prey population (Benard, 2004; Bourdeau & Johansson, 2012; Davenport et al., 2014). Predators differ in hunting and pursuing features, and densities among habitats (Mcpeck, 1990b; Wellborn et al., 1996). Thus, changes in direction and strength of selection induced by different predators are the major driver of prey population divergence (Franks & Oxford, 2017; Herczeg et al., 2009). Previous work on population divergence has mainly focused on the changes in trait mean (Violle et al., 2012), with data on changes in trait variances in response to different predators being scarce (see Garamszegi & Møller, 2017; Poléo et al., 1995; Runemark et al., 2014). However, as a prime target of natural selection, differences in trait variances among populations might indicate incipient speciation (Gosden et al., 2011; Tregenza et al., 2000), and unveil the relationship between phenotypic variation and fitness (Nosil & Crespi, 2006). Thus, combined changes in trait mean and trait variance can inform us about the evolutionary dynamics of the population.

Freshwater habitats offer a great opportunity to study trait dynamics in population divergence because habitats typically vary in the top predator assemblages. Permanent ponds and lakes are characterised by either the occurrence of predatory fish (hereafter called fish lakes) or the occurrence of large predatory invertebrates (hereafter called dragonfly lakes) (Wellborn et al., 1996). Both predator species differ in prey detection, pursuing and capturing features, resulting in diversifying selection of prey (Benard, 2004). Thus, selection by different predation regimes is responsible for the differential occurrence of prey species along the freshwater habitat gradient (Sih et al., 1985; Stroud & Losos, 2016), and this selection can also cause population divergence within prey species (Blumstein & Daniel, 2005; Dahl & Peckarsky, 2002; Magalhaes et al., 2016).

However, the selection of antipredator traits by predatory fish and predatory invertebrates can be context-dependent (influenced by complex factors). Predatory fish select for long abdominal spines (dragonfly larvae in Johansson & Mikolajewski, 2008, stickleback in Miller et al., 2017), whereas antagonistic selection by predatory invertebrates results in reduced spine length (Mikolajewski et al., 2006). Such directional selection could result in different overall means between fish-lake and dragonfly-lake populations (Bashevkin et al., 2020; Johansson, 2002). However, patterns in trait variance might be more complex. Production of defensive spines likely comes at a significant energetic cost (Flenner et al., 2009; Mikolajewski & Johansson, 2004). Thus, individuals might be able to save costs by

developing shorter spines but still survive because of habitat complexity via for example, plant cover providing shelter (Henrikson, 1993; Thomaz & Cunha, 2010). Individuals living in plant cover are also more likely to face invertebrate predators (Brenda, 1995) which select for short defensive spines (see above), though invertebrate predators occur in a very small number of fish lakes (Mcpeck, 1990b). In contrast, individuals in dragonfly lakes will develop shorter spines not only because they can save the energetic costs of spine production, but also because invertebrate predators select against spines. This might result in stronger stabilising selection for short spines in dragonfly-lake populations than for long spines in fish-lake populations, resulting in a lower variance in dragonfly-lake populations than in fish-lake populations.

Body size represents a key phenotypic trait affecting most aspects of an individual and its relationship with other organisms (Blanckenhorn, 2000; Dmitriew, 2011). Body size is under considerable selection by predators (Schmidt & Van Buskirk, 2005; Warren & Lawton, 1987; Ziemba et al., 2000) and usually scales with growth rate (Peacor et al., 2007; Peters, 1986; Relyea, 2001). In cases where predation risk is size-dependent, shifts in the mean body size of preys among different predation regimes can be expected if predators favour different prey sizes (Price et al., 2015). Variation in body size can increase, for example, predator-mediated behavioural changes differing among predator types, because of scaling effects of growth rate with body size (Peacor et al., 2007; Uchmański, 1985). For instance, variation in body size is predicted to increase, if larger individuals, that are safe from predation, will proportionally grow more than smaller and more vulnerable individuals because size-dependent behaviours change food availability and subsequently growth rate (Eklöv & Werner, 2000). In contrast, we can also expect shifts in mean body size, if predators cause different selective strength on foraging behaviour (Ercit, 2016; Reznick, 1982). In this case, body size variation is predicted to be lower under a stronger reduction in predator-mediated behavioural changes, because decreased food intake leads to reduced growth and subsequently decreased individual variations (Peacor et al., 2007; Ziemba et al., 2000).

Here, we study changes in mean and variance in larval defensive spines and body size among populations of the dragonfly *Leucorrhinia pectoralis* (Charpentier, 1825). Species of the genus *Leucorrhinia* separate strongly between fish lakes and dragonfly lakes (Hovmöller & Johansson, 2004; Petrin et al., 2010), whereas some species including *L. pectoralis* can occur in both fish lakes and dragonfly lakes (Johansson & Brodin, 2003; Mikolajewski, Scharnweber, et al., 2016; Petrin et al., 2010). Thereby, intraspecific trait divergence should be similar to interspecific trait divergence in its magnitude and direction (Mikolajewski et al., 2010; Mikolajewski, Scharnweber, et al., 2016). Larval *Leucorrhinia* in fish lakes develops dominant abdominal spines that protect them against predatory fish (Mikolajewski & Rolff, 2004; Johansson & Mikolajewski, 2008), whereas antagonistic selection by predatory invertebrates results in the reduction of spine length (Mikolajewski et al., 2006). Based on these patterns, we hypothesise (i) fish-lake populations possess longer abdominal spines than dragonfly-lake populations. Furthermore, because of the above-

TABLE 1 Sampled populations of *Leucorrhinia pectoralis* (represented by sampling location) with information of sampling year, predation regime of each sampling lake (lake type), number of exuviae per year (sample size), and the GPS coordinates

Sampling location	Sampling year	Lake type	Sample size	Coordinates
Wetzikon	2012/2013	Dragonfly lake	29/60	47° 18' N 8° 47' E
Binsenmoor	2008	Dragonfly lake	22	53° 26' N 13° 47' E
Brennbruch	2003/2005/2006	Fish lake	4/18/2	53° 23' N 13° 36' E
Bruesenwalde	2004/2013	Dragonfly lake	23/58	53° 26' N 13° 47' E
Buckowsee	2005	Fish lake	16	53° 00' N 13° 88' E
Dreiecksee	2013	Fish lake	3	53° 20' N 13° 93' E
Fl. Cloewensee	2012	Fish lake	84	53° 23' N 13° 51' E
Gartenweiher Neuhaus	2001	Fish lake	9	53° 08' N 13° 83' E
Gr. Borgsee	2006/2007/2008	Fish lake	2/7/39	53° 19' N 13° 95' E
Gr. Krinertsee	2011	Fish lake	11	53° 08' N 13° 73' E
Gr. Mehlitzsee	2008	Fish lake	4	53° 08' N 13° 63' E
Gr. Barschsee	2002/2004	Fish lake	10/3	53° 11' N 13° 00' E
Roetseemoos	2012	Dragonfly lake	19	47° 49' N 9° 53' E
Herzfelde Sölle	2009	Dragonfly lake	36	53° 20' N 13° 59' E
Kesselmoor	1996/1997	Dragonfly lake	57/81	53° 16' N 13° 91' E
Kl. Baberowsee	2002/2011	Fish lake	1/7	53° 24' N 13° 48' E
Kl. Griebchen	1998/2003	Fish lake	6/1	53° 16' N 13° 42' E
Kl. Maeuschensee	2013	Fish lake	50	53° 20' N 13° 58' E
Kl. Toernsee	2002	Fish lake	26	53° 10' N 13° 00' E
Suckowsee	2001	Fish lake	86	53° 25' N 13° 59' E
Knehdenmoor	2008	Dragonfly lake	6	53° 14' N 13° 54' E
Koelpinmoor	2011	Dragonfly lake	48	53° 11' N 13° 69' E
Krummer See	2009/2011/2012/2013/2015	Fish lake	13/33/8/27/15	53° 16' N 13° 91' E
Laatzer See	2003/2004	Fish lake	4/6	53° 13' N 13° 59' E
Lange Wiese	2009/2012	Dragonfly lake	5/14	53° 23' N 13° 42' E
Lehst-Niederung	2006	Fish lake	16	53° 22' N 13° 34' E
Madlener Moos	2012	Dragonfly lake	19	47° 44' N 9° 43' E
Mellenmoor	1998	Dragonfly lake	18	53° 19' N 13° 29' E
Mittlerer Pöhl	2001	Dragonfly lake	108	53° 29' N 13° 12' E
Poviestsee Moor	2008	Dragonfly lake	6	53° 23' N 13° 51' E
Zahrensee Moor	2009	Dragonfly lake	8	53° 24' N 13° 21' E
Redernswalde Moor	1990	Dragonfly lake	27	53° 04' N 13° 84' E
Warthe Moorkolk	2002/2003/2005/2006	Fish lake	34/7/16/9	53° 24' N 13° 49' E
Moosbruch	2004/2005	Dragonfly lake	3/5	53° 24' N 13° 38' E
Oberpfuhlmoor	2006/2012	Dragonfly lake	7/9	53° 21' N 13° 33' E
Pfingstposse	1998	Dragonfly lake	6	53° 00' N 13° 92' E
Poviestsee	2013	Fish lake	89	53° 23' N 13° 51' E
Quellmoor	2010	Dragonfly lake	34	53° 24' N 13° 39' E
Reichermoos	2012	Dragonfly lake	14	47° 46' N 9° 44' E
Obermooweiler	2012	Dragonfly lake	19	47° 39' N 9° 47' E
Steinacher Ried	2012	Dragonfly lake	20	47° 55' N 9° 42' E
Steißsee	2000/2007/2013	Fish lake	18/2/11	53° 17' N 13° 51' E
Teufelsbruch	2011	Dragonfly lake	47	53° 17' N 12° 92' E
Thomsdorf Moor	2012	Dragonfly lake	45	53° 26' N 13° 47' E
Torfbruch Densow	2013	Dragonfly lake	54	53° 15' N 13° 39' E
Torfstich Schnakenpfuhl	2010/2013	Dragonfly lake	5/14	53° 24' N 13° 37' E

Note: Ponds and lakes are characterised by either the occurrence of predatory fish (fish lakes) or the occurrence of large invertebrate predators (dragonfly lakes).

described relationship of different costs and benefits in spine length between fish-lake and dragonfly-lake populations, we hypothesise that (ii) fish-lake populations will exhibit larger variations in spine length than dragonfly-lake populations. However, the relationship of body size in relation to both predation regimes is less well studied. Data from other species indicate that prey might become safer with increasing body size regardless of the predator (Mikolajewski & Johansson, 2004; Stoks et al., 2012). There is also evidence that larval *Leucorrhinia* from dragonfly lakes forage more (Jiang & Mikolajewski, 2018), but that growth rates do not differ because of compensatory physiological mechanisms (Jiang et al., 2019). Consequently, we hypothesise (iii) no differences in mean body size between fish-lake populations and dragonfly-lake populations. Furthermore, because growth rate and food intake were similar in experimental trials among larval *Leucorrhinia* from fish lakes and dragonfly lakes, we hypothesise (iv) no difference in body size variance among fish-lake and dragonfly-lake populations.

MATERIALS AND METHOD

Sample collection

We collected exuviae of *L. pectoralis* from 46 sites (permanent lakes, ponds, bogs, etc.) across Germany and Switzerland (Table 1). Exuviae of last instar larvae were collected on emergent vegetation along the shoreline. Shorelines represent typical odonate emergence habitats. From 1990 to 2015, exuviae were collected from mid-May to the end of May which is the main emergence period of *L. pectoralis* (Sternberg & Buchwald, 1999). Exuviae were stored under dry conditions and categorised by collection site and year at room temperature; the sample size for each collection site and year is presented in Table 1.

Collection sites differed in the occurrence of predators, with predatory fish either present or absent. The presence of predatory fish was determined during the sampling period by fishing or based on the data provided by local fishermen, angling societies, and provincial environmental authorities. The common species present were crucian carp (*Carassius carassius*, Linnaeus, 1758), pike (*Esox lucius*, Linnaeus, 1758), perch (*Perca fluviatilis*, Linnaeus, 1758), common roach (*Rutilus rutilus*, Linnaeus, 1758), and common rudd (*Scardinius erythrophthalmus*, Linnaeus, 1758). In sites without predatory fish, common large invertebrate predators were recorded by net sampling. The collecting samples of invertebrate predators included exuviae and dead individuals of dragonfly larvae (Aeshnidae), larvae of the beetle (*Dytiscus* sp.), and the backswimmer (*Notonecta* sp.).

Spine length and body size measurements

We took digital photographs of each exuvia from the dorsal, ventral, and lateral sides using an Olympus digital microscope SZX16 (Hamburg, Germany). We measured 1623 exuviae (926 exuviae from 26 dragonfly lakes and 697 exuviae from 20 fish lakes). The abdominal

TABLE 2 Loadings, eigenvalues and variance explained by each PC based upon covariance matrix of the first two principal components (PC1-PC2)

Measurement	PC1 (spine length)	PC2 (body size)
Dorsal spine 5	0.79	0.13
Dorsal spine 6	0.88	0.09
Dorsal spine 7	0.89	0.09
Dorsal spine 8	0.85	0.11
Lateral spine 8	0.8	0.22
Lateral spine 9	0.82	0.27
Pro-femur	0.16	0.73
Pro-tibia	0.17	0.8
Meso-femur	0.15	0.84
Meso-tibia	0.15	0.86
Meta-femur	0.14	0.85
Meta-tibia	0.19	0.86
Eigenvalue	5.87	2.77
Variance explained	0.36	0.36

Note: Factor loadings in bold indicate variables loading highly on each axis (loadings >0.70).

spine length and body size were determined from photographs using the free software ImageJ 1.50 g (National Institutes of Health, USA 2016) (Schneider et al., 2012).

Abdominal spine length

The length of the dorsal posterior spines at the abdominal segments 5–8 and lateral spines at abdominal segments 8 and 9 were measured from the base to the tip of the spines (Johansson & Samuelsson, 1994). We did not measure dorsal anterior abdominal spines as those were covered by wing pads and were probably not under selection by predators (Petrin et al., 2010).

Body size

Head width represents the most common body size surrogate in odonate larvae (Benke, 1970). However, head width cannot be measured in exuviae because the head capsule is split-open from emergence. Therefore, we used the length of the pro-, meso-, and meta- femur and tibia (Falck & Johansson, 2000; Petrin et al., 2010) as surrogates for body size. Measurements of only the right femur and tibia were used.

Statistical analyses

All analyses were performed using R 4.0.5 (R Core Team, 2021). Because of the high multicollinearity among the measurements of abdominal spines and body size, we first performed principal

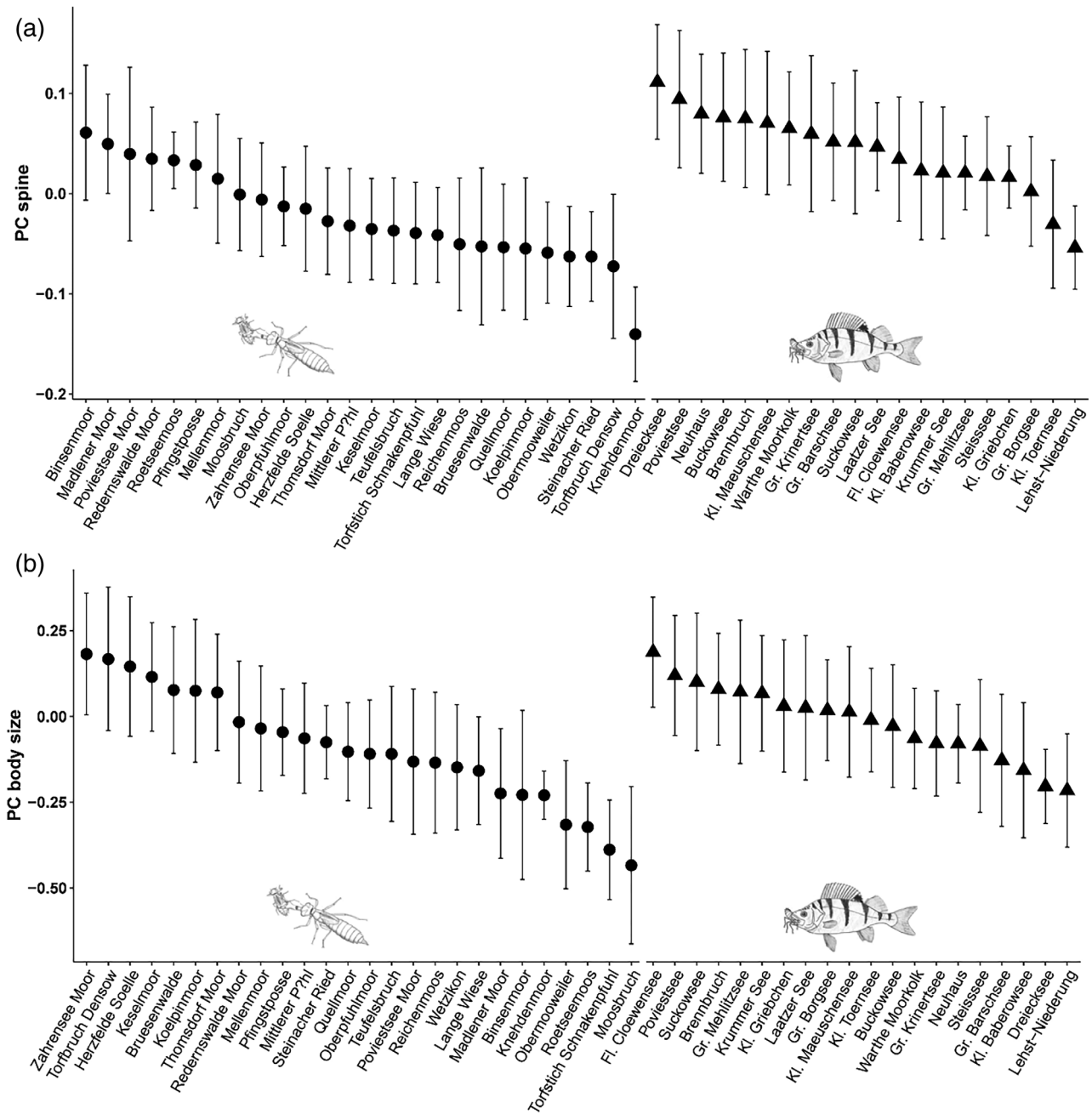


FIGURE 1 Means \pm SD of (a) spine length (PC spine) and (b) body size (PC body size) of each sampled dragonfly-lake (circle) and fish-lake (triangle) population. Fish and dragonfly larvae symbols also indicate different predation regimes for populations. For the sample size, please refer to Table 1

component analyses (PCA, package ‘psych’, [Revelle, 2016]) on the entire dataset using a covariance matrix to reduce the number of variables. The PCA extracted two principal components (PC), which explained 72% of the variance, with body size surrogates loading high on PC1 (PC Body size, Table 2). PC2 captured the variance from all abdominal spine measurements (PC Spine, Table 2). The two extracted principal components were used in subsequent analyses.

To evaluate differences between fish-lake and dragonfly-lake populations in trait means of abdominal spine length (PC Spine) and body size (PC Body size), we constructed linear mixed effect models (packages ‘lme4’, [Bates, 2010]) using predation regimes (fish-lake

vs. dragonfly-lake) as a fixed effect and sampling year, as well as, the sampling location nested in predation regimes as random effects. We did not include body size in the model for spine length because they were not correlated which is also supported by the fact that PC Spine and PC Body size were orthogonal to each other.

Levene’s tests were conducted to determine the differences in trait variance of abdominal spine length (PC Spine) and body size (PC Body size) between dragonfly-lake populations and fish-lake populations. To determine whether total variance was greater among fish-lake populations than among dragonfly-lake populations, we applied Levene’s test across fish-lake and dragonfly-lake populations.

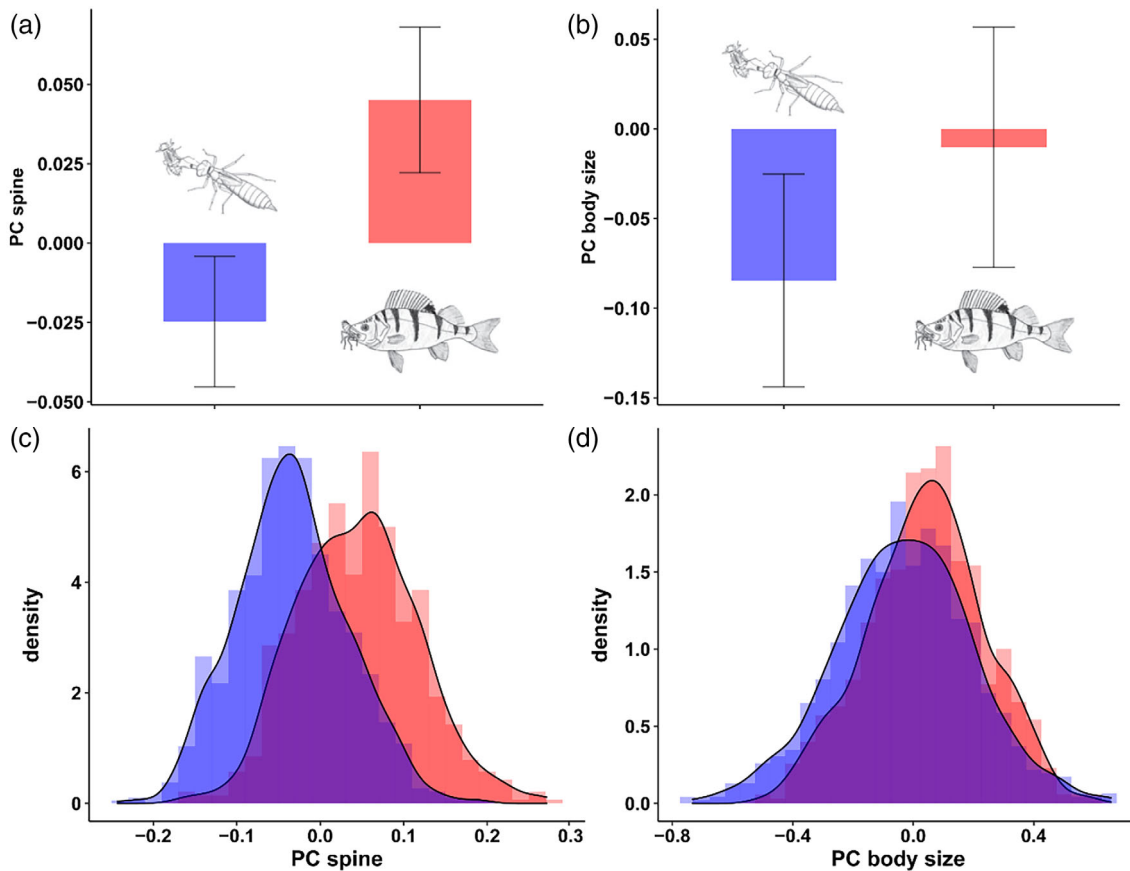


FIGURE 2 Estimated marginal means ($\pm 95\%$ confidence intervals) for (a) spine length (PC spine) and (b) body size (PC body size) as well as frequency distributions of (c) spine length (PC spine) and (d) body size (PC body size) for larvae from fish lakes indicated in red and from dragonfly lakes indicated in blue. Fish and dragonfly larvae symbols indicate different predation regimes for populations

To determine whether interpopulation variances had the same pattern as total variances, we first calculated the mean of PC Spine and PC body size of each population. Then, we conducted Levene's test with those mean estimates to compare the interpopulation variances across fish-lake and dragonfly-lake populations. To visualise variances components between fish-lake and dragonfly-lake populations, we grouped our data into subsets based on predation regimes. Linear mixed models were constructed with the population as the fixed effect and sampling time as the random effect to decompose total variances into between-population and within-population variances.

RESULTS

Trait mean differences between fish-lake and dragonfly-lake populations

Populations from fish lakes had longer dorsal and lateral abdominal spines than those from dragonfly lakes (PC Spine: $\chi^2 = 26.75$, d.f. = 1, $p < 0.001$) (Figures 1a and 2a). There was no significant difference for body size between fish-lake populations and dragonfly-lake populations (PC body size: $\chi^2 = 3.21$, d.f. = 1, $p = 0.07$) (Figures 1b and 2b).

Trait variances differences between fish-lake and dragonfly-lake populations

1. *Total variance*: Total variance for spine length was significantly larger in fish-lake populations than that in dragonfly-lake populations (PC Spine: $F_{1,1619} = 6.95$, $p = 0.008$; Figure 3a). Total variance for body size was significantly smaller in fish-lake populations than that in dragonfly-lake populations (PC body size: $F_{1,1619} = 14.74$, $p < 0.001$; Figure 3b).
2. *Interpopulation variance*: Interpopulation variances for spine length (PC Spine: $F_{1,44} = 0.10$, $p = 0.757$) and body size (PC body size: $F_{1,44} = 3.40$, $p = 0.072$) did not differ between fish-lake and dragonfly-lake populations (Figure 3a,b).

DISCUSSION

In this study, we found that *L. pectoralis* larvae from dragonfly lakes had shorter spines than those from fish lakes. Moreover, we also found that the dragonfly-lake populations of *L. pectoralis* had a smaller total variance in spine length compared to that of fish-lake populations. However, they had a larger variance in body size than

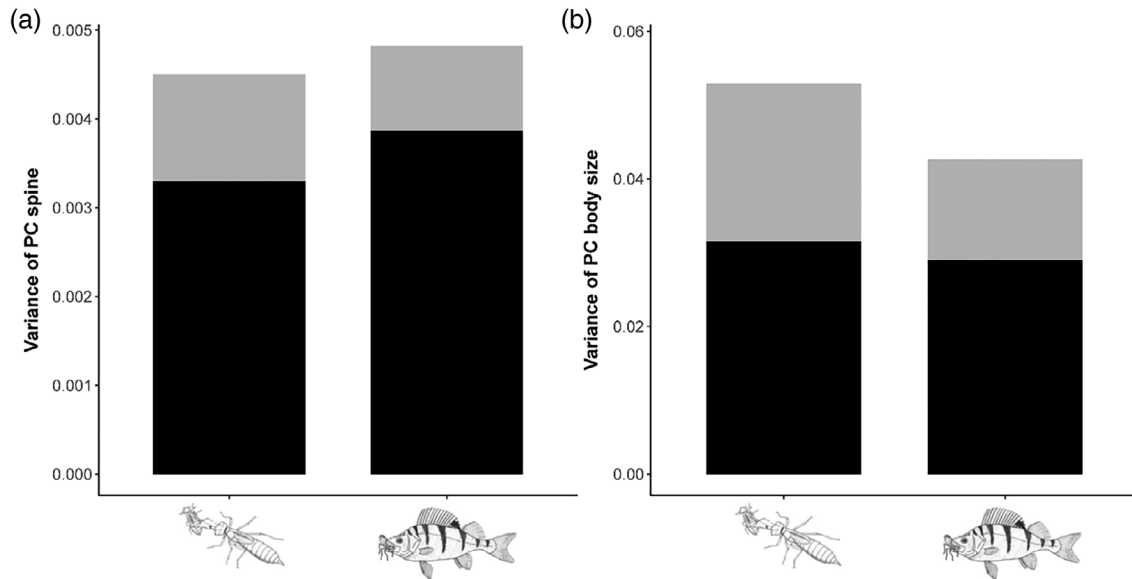


FIGURE 3 Variance decompositions of (a) spine length (PC spine) and (b) body size (PC body size) between larvae from dragonfly lakes and fish lakes (indicated by drawings). Light grey indicates between-population variances and black indicates within-population variances

that of fish-lake populations. Our results suggested that both means and variances of antipredator traits and body size were under strong selection by different predators, causing the population to diverge. However, the strength and direction of selection were predator-specific.

In aquatic systems, the long spines of prey provide protection against gape-limited predatory fish (Bashevkin et al., 2020; Johansson & Mikolajewski, 2008; Price et al., 2015; Riessen & Sprules, 1990; Šigutová et al., 2018; Zhang et al., 2017). Here, we showed that the larvae of *L. pectoralis* also expressed longer spines in fish lakes than in dragonfly lakes. Similar interspecific patterns were observed within the genus *Leucorrhinia*: larval abdominal spines of fish-lake species are longer than those of dragonfly-lake species (reviewed in Johansson & Mikolajewski, 2008). Furthermore, intraspecifically, two *Leucorrhinia* species (*L. dubia* and *L. intacta*) plastically exhibited long spines in fish lakes but short spines in dragonfly lakes (Johansson, 2002; McCauley et al., 2008). Thus, predatory fish impose strong selection for long spines in dragonfly larvae, while invertebrate predators select against long spines at interspecific and intraspecific levels. Additionally, we found that spine length had a higher total variance in fish-lake populations than in dragonfly-lake populations, and this was entirely driven by a reduction in within-population variance among dragonfly-lake populations. A reduction in the variance in dragonfly-lake populations might be due to stabilising selection (Lahti et al., 2009). Long spines are detrimental to prey in dragonfly lakes because invertebrate predators can more easily hold on to long-spined individuals (Mikolajewski et al., 2006). Therefore, high costs are expected when larvae have long spines in dragonfly lakes. The spine length in fish-lake populations had a larger variance, and this result fits our early hypothesis. In fish lakes, a larger variance in spine length could result from weaker stabilising selection, which usually leads to a broader fitness peak of traits (Lahti et al., 2009).

Selection pressures on spine length in fish lakes might differ due to different habitat complexity, size, and density heterogeneity of predatory fish, and so forth. Therefore, by living in habitats with different levels of selection pressures, *L. pectoralis* larvae with differences in the spine length might survive predatory fish.

Although both body size and spines are related to the fitness of prey, they showed different patterns of diversification under different predation regimes. The body size of the fish-lake and dragonfly-lake populations did not differ in *L. pectoralis*. This is not surprising because previous studies found that *L. pectoralis* did not reduce its activity in response to predatory fish, and thus, they cannot acquire more food for growth via increasing activity in dragonfly lakes (Mikolajewski, Conrad, et al., 2016). In fish lakes, food for dragonfly larvae, such as zooplankton and damselfly larvae, decreases at the species level (taxonomic richness) and the population level (overall biomass) because fish are upper-level consumers (Gophen, 2017; Laske et al., 2017; Reissig et al., 2006). Therefore, predatory fish may decrease the food availability and food competitors for *L. pectoralis*, simultaneously. Thus, the food resources for each individual may remain unchanged. Moreover, in fish lakes *L. pectoralis* are semivoltine, and in dragonfly lakes, they are univoltine or semivoltine (Mauersberger & Mikolajewski pers. observation). Differences in the duration of the larval period may further reduce the differences in the mean of the body size of the last instar larvae, while a longer time spent in the larval stage in fish-lake populations might also minimise the body size variance of the last instar larvae.

Unfortunately, we could not determine if differences in trait means and changes in trait variances between fish-lake and dragonfly-lake populations were caused by genetic divergence or mediated by phenotypic plasticity. It has been shown that both factors alter the trait mean and variance (Colosimo et al., 2004; Robinson et al., 2015; Runemark et al., 2014). Mikolajewski, Conrad, et al. (2016) showed

that *L. pectoralis* larvae lack plastic antipredator behaviour by comparing the absence/presence of predatory fish. However, phenotypic plasticity in response to predation was shown in *L. dubia* and *L. intacta* for spine length (Johansson & Samuelsson, 1994; McCauley et al., 2008), and in *L. dubia* and *L. albifrons* for behaviour (Mikolajewski & Johansson, 2004); however, phenotypic plasticity was absent in *L. dubia* for body size (Johansson, 2002). These observations suggest a potential role of phenotypic plasticity in the patterns that we found. Furthermore, long-distance flight in dragonfly species like *L. dubia* (Pajunen, 1962) might lead to weak genetic differentiation among populations (Johansson, Halvarsson, Mikolajewski, & Högglund, 2017). However, variation in the spine length was found in several *Leucorrhinia* species by comparing fish-lake and dragonfly-lake populations (Petrin et al., 2010). Additionally, recent common garden experiments excluding phenotypic plasticity revealed apparent genetic divergence among Palearctic populations in the spine length of *L. dubia* (Johansson, Halvarsson, Mikolajewski, & Högglund, 2017). Thus, common garden experiments are necessary to disentangle the effects of phenotypic plasticity and genetic differentiation on the changes in the mean and variance of the defensive spine length and the body size across fish-lake and dragonfly-lake populations.

Interpopulation variance for spine length or body size did not differ significantly between fish-lake and dragonfly-lake populations. This indicated that in fish and dragonfly lakes, the strength and direction of selection are comparable across populations. This is surprising because different fish and dragonfly lakes differ in chemical factors, size, vegetation, and so forth. For instance, water chemistry and predator composition are quite different across lakes (Mcpeck, 1990b); *Sphagnum* mosses provide hiding spots for *L. dubia* to avoid fish predators, and thus, these mosses can alter the microhabitat for dragonfly larvae in different lakes (Henrikson, 1993). However, predation is a major selective force driving the occurrence of dragonfly larvae, while other abiotic factors have relatively little impact on the occurrence of dragonfly larvae (Johansson & Brodin, 2003; Mcpeck, 1990b). Therefore, predation pressures may overwhelmingly dominate the selection pressure on the population differentiation of dragonfly larvae.

Studies on the phenotypic selection of quantitative traits in the wild are urgently needed (Kingsolver & Diamond, 2011). The special life history of the dragonfly species, such as strong dispersal ability in adults and isolation of larvae in lakes, could provide an opportunity to disentangle phenotypic selection mechanisms (Bybee et al., 2016). In this study, we found that *L. pectoralis* larvae from dragonfly-lake populations had shorter spines than the larvae from fish-lake populations. The selection pressure imposed by invertebrate predators also reduced the variance of the spines in *L. pectoralis* in contrast to the selection pressure imposed by predatory fish. These results highlighted that intraspecific divergence could be as strong as interspecific divergence (interspecific divergence in spines shown in [Hovmöller & Johansson, 2004, Johansson & Mikolajewski, 2008]). The changes in the mean and variance of the length of antipredator spines suggested directional and stabilising selection in the *Leucorrhinia*-predator system (Kingsolver et al., 2012; Lande & Arnold, 1983). Furthermore, by systematically investigating two

antipredator traits, we found that different antipredator traits may respond differently to various predation systems. Selection pressures on prey traits by top predators can be influenced by complex factors.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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