The Journal of Experimental Biology 215, 3453-3458

doi:10.1242/jeb.071142

RESEARCH ARTICLE

The proximal costs of case construction in caddisflies: antioxidant and life history responses

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SUMMARY

Animal construction allows organisms to cope with environmental variations but the physiological costs of such behaviour are still poorly understood. The aim of the present study was to measure the physiological cost of construction behaviour through the oxidative balance that is known to affect the ability of organs to function, stimulates senescence processes and ultimately impacts the fitness of the organism. We used larvae of caddisfly, *Limnephilus rhombicus*, by experimentally modifying the effort associated with case building. Larvae that were forced to build a new case showed a significant increase in both total antioxidant capacity and the specific activity of superoxide dismutase 48 and 72 h, respectively, after the initiation of the reconstruction. These results strongly suggest that the larval construction behaviour triggered the production of reactive oxygen species, but their effects were reversed 7 days after the reconstruction. In the animals that were forced to build a new case, oxidative stress appeared to be mitigated by a network of antioxidant defences because no oxidative damage was observed in proteins compared with the control larvae. At the adult stage, while longevity was not sex dependent and was not affected by the treatment, body mass and body size of adult males from the reconstruction treatment were significantly lower than the control values. This unexpected sex effect together with data on oxidative stress highlights the difficulty of determining the physiological cost associated with energy-demanding behaviours, implying a consideration of both their energetic and non-energetic components is required.

Key words: *Limnephilus rhombicus*, longevity, morphology, oxidative stress, protein carbonyl content, superoxide dismutase.

Received 13 February 2012; Accepted 19 June 2012

INTRODUCTION

Behaviour allows organisms to cope with physical and biotic variations in their environment. If the benefits of behaviour are evident and well documented (e.g. Jaloux et al., 2005; Lengagne and Joly, 2010), understanding of the fitness impacts of an organism's behavioural adaptation requires investigation of the associated costs. For over 40 years it has been supposed that physiological costs are caused by the allocation of limited resources among competing traits such as maintenance, survival and reproduction (Levins, 1968; Stearns, 1992). However, many researchers suggest the need to incorporate the costs of non-energetic mechanisms, such as endocrine regulation or oxidative status, in the measurement of physiological cost (reviewed in Zera and Harshman, 2001).

In the past 10 years, reactive oxygen species (ROS) have been identified as an important proximal parameter of resource allocation (Monaghan et al., 2009). This result is the basis of the concept of oxidative stress ecology (McGraw et al., 2010). The behaviours that require energy increase metabolic rate and stimulate ATP-generating processes within the mitochondria (oxidative phosphorylation), and, therefore, are likely to increase the production of harmful oxygen-centred free radicals (or ROS). Indeed, the rate at which ROS are generated during oxidative phosphorylation is frequently estimated as 0.1–3% of the inspired oxygen (Golden and Melov, 2001; St Pierre et al., 2002). Although low levels of ROS are important in cell signalling and in the induction of host defence genes (reviewed in Dowling and Simmons, 2009; Costantini et al., 2010), the excessive release of ROS is known to induce damage to lipids, proteins and DNA, resulting in oxidative stress, loss of cell function and programmed cell death (reviewed in Dowling and Simmons, 2009). To moderate the continuous production of pro-oxidant molecules by the mitochondria, ROS are degraded by a complex array of antioxidant systems. These systems include inducible enzymes, such as superoxide dismutase (SOD), glutathione peroxidase and catalase, and low-molecular weight molecules, such as vitamin A, C, D or E, thioredoxin, glutathione, carotenoids or uric acids (reviewed in Dowling and Simmons, 2009). Oxidative stress is usually observed if the rate of ROS production impairs the antioxidant defences. Oxidative stress, antioxidant production and the damaging effect of oxidative stress may produce important physiological costs and affect growth, longevity, reproduction and the immune response (Costantini, 2008; Dowling and Simmons, 2009; Costantini et al., 2010; Metcalfe and Alonso-Alvarez, 2010; Geiger et al., 2011) (but see Selman et al., 2008; Garratt et al., 2011).

Construction behaviour occurs in a large number of animals and allows an extension of control over some aspects of the environment (reviewed in Hansell, 2005). These constructions can protect the animals against predators (e.g. Endler, 1981), help to control

physical parameters (e.g. Strahl et al., 2011), serve to catch prey (e.g. Tseng et al., 2011) and/or more rarely serve as signals (reviewed in Schaedelin and Taborsky, 2009). Several studies have already evaluated the cost of secretion of the material from which the structures are made (Eisner, 1994; Stevens et al., 1999; McKie, 2004; Hansell, 2005; Mondy et al., 2011) and the energetic costs, which include metabolic energy expended gathering materials and building the structure (Abarca and Boege, 2011; Mondy et al., 2011). However, the non-energetic costs of construction behaviour remain largely unknown. Caddisfly species provide a suitable biological model for studying the physiological costs linked to construction behaviour and their impact on the life history strategy. Indeed, the aquatic larvae of many caddisfly species build their own portable cases made of organic and/or mineral particles cemented with the silk that they secrete (Hickin, 1967). Trichopteran case loss and reconstruction can occur naturally and relatively often as a response to the attacks of predators, such as dragonfly larvae or salamanders (e.g. Johansson and Johansson, 1992; Jannot et al., 2007). Because most caddisfly adults feed minimally or not at all (Mosely, 1939), the acquisition of resources during the larval stage is crucial for growth, case construction, reproduction and maintenance. Several studies have shown that larvae forced to rebuild a case experience a severe increase in energy expenditure, and such an increase can impact the dispersal and reproductive capacities of the adults [Caddisfly (Stevens et al., 1999; Stevens et al., 2000; Jannot et al., 2007), Chironomid (McKie, 2004)]. In a previous study, we found that larvae of *Limnephilus rhombicus* Linnaeus (Trichoptera: Limnephilidae) forced to rebuild a case increased their oxygen consumption by 50% during the period of reconstruction, compared with control larvae (Mondy et al., 2011). The higher metabolic demands of reconstruction were associated with a dramatic reduction of total body protein content in both larvae and adults (Mondy et al., 2011).

In this paper, we report the results of an experimental study aiming to measure the oxidative cost of construction in the caddisfly *L. rhombicus*. We predicted that the larval stimulation of aerobic metabolism during case construction would increase ROS production, leading to an oxidative challenge that may in turn trigger a compensatory response. To cope with the high level of oxidant molecule generation, two separate scenarios may occur. First, larvae may up-regulate their antioxidant defences in response to increased ROS to prevent oxidative damage. If this scenario is accurate, we might observe little or no effect of reconstruction behaviour on oxidative stress markers, and adult longevity may not be affected. However, because of the amount of energetic resources invested in defence, morphological traits, such as body size or the total or regional body mass of the adult, might be affected by reconstruction occurring at the larval stage. In contrast, a second scenario may result if no energy is allocated to antioxidant defences. As a consequence, we might observe a clear increase of oxidative damage in larvae performing reconstruction and a subsequent effect on adult longevity. We experimentally forced *L. rhombicus* larvae to rebuild a case and assessed both the antioxidant response and oxidative markers. Morphology and longevity parameters were measured in the adults that emerged from the treated and control larvae.

MATERIALS AND METHODS

Animals and experimental design

Three-hundred sixty-fifth stage larvae of *L. rhombicus* were collected in May 2011 from a natural population in Annoisin pond in southeastern France (45°75′N, 5°28′33″E). Larvae develop in slow-running brooks and in still-water habitats where oxygen levels are low. In Annoisin pound, measurements taken during the collection of larvae showed daily variations in water oxygen content ranging from 2 to 14 mg l^{-1}. The size of each head capsule was measured upon capture. The larvae were housed in plastic boxes (18×22×17 cm height×length×width) containing 500 ml of water (20–25°C, 6–9 mg l^{-1} of oxygen). Five larvae were placed in each box. The larvae were fed with *Myriophyllum verticillatum* and were reared under a natural photoperiod. The water and plants were renewed once a week.

Larvae were randomly assigned to two groups, namely ‘control’ and ‘treated’ groups. Thirty larvae from each group were collected and frozen at –80°C for subsequent analysis (see below), allowing us to check the homogeneity between these two groups. All the other larvae were removed from their cases at 16:00 h on the first day of the experiment (0 h). One-hundred and eighty control larvae were returned to their original cases, whereas the other 180 larvae, the treated larvae, were forced to build a new case. To evaluate the oxidative stress resulting from the case reconstruction, 20 larvae from each group (control *versus* treated) were randomly collected at different times: 48 h after case elimination (48 h), 72 h after case elimination (72 h) and 1 week after case elimination (7 days). The larvae were immediately weighed and frozen at –80°C until used. To measure different life history traits of adults (longevity, body size and allocation of body mass), adults were also collected and sexed on the day of their emergence (see below).

Measurement of oxidative stress markers

Antioxidant defences

The total antioxidant capacity and the specific enzymatic activity of SOD were measured in tissue homogenates extracted from 10 control larvae and 10 treated larvae randomly collected at each of the following times: 0 h, 48 h, 72 h and 7 days from the beginning of the experiment. The insects were crushed individually with high-speed shaking in a Tissue Lyser (Retsch, Haan, Germany) for 30 s at 30 Hz in 2 ml Eppendorf tubes containing stainless steel beads and 600 µl of extraction buffer (100 mmol l^{-1} KH_2PO_4, 1 mmol l^{-1} DTT, 2 mmol l^{-1} EGTA, pH adjusted to 7.4 at 4°C with NaOH). Each tube was then supplemented with 600 µl of extraction buffer (thus reaching a final volume of 1.2 ml) and centrifuged at 10,000 ***g*** for 5 min at 4°C.

The total antioxidant capacity was measured using Cayman’s antioxidant assay kit (no. 709001, Cayman Chemical Company, Ann Arbor, MI, USA), combining the activities of all the antioxidant constituents. The assay relies on the ability of the antioxidants in the sample to inhibit the oxidation of ABTS [2,2′-Azino-di-(3-ethylbenthiazoline sulphonate)] to $ABTS^+$ by metmyoglobin. The amount of $ABTS^+$ produced was detected spectrophotometrically at 405 nm (BioTek EL808, Winooski, VT, USA). The capacity of antioxidants in the sample to prevent the oxidation of ABTS was compared with that of Trolox, a water-soluble tocopherol analogue. The results are expressed as Trolox-equivalent antioxidant capacity (TEAC). The total SOD activity (Cu/Zn, Mn and FeSOD) was determined with spectrophotometry at 550 nm through the use of the xanthine and xanthine oxidase systems (superoxide dismutase assay kit no. 706002, Cayman Chemical Company). One unit of SOD activity was defined as the mass of enzyme needed to exhibit 50% dismutation of the superoxide radical.

Protein carbonyl content

We chose to measure the protein carbonyl content as an index of oxidative injuries for the following reasons: (i) oxidative damage

to proteins is considered a key indicator of oxidative stress (Dalle-Donne et al., 2003); (ii) the total protein content of larvae and adults of *L. rhombicus* was impacted by the construction behaviour as a result of the production of silk needed to build a case (Mondy et al., 2011); and (iii) previous studies on insects showed, at the inter- and intra-specific level, that protein carbonyl content is associated with life expectancy (Sohal et al., 1993; Sohal et al., 1995). This biomarker has been widely employed to estimate oxidative damage in different animal models, including insects (Sohal et al., 1993; Sohal et al., 1995). The larvae were collected in the same way as for antioxidant determination (N=10 control, N=10 treated, at each sampling time during the experiment). The protocol for protein carbonyl extraction was similar to that used for antioxidant extraction. The oxidative damage to proteins was measured with the Protein Carbonyl Assay kit (no. 10005020, Cayman Chemical Company) using 2,4-dinitrophenylhydrazine (DNPH) reagent. DNPH reacts with protein carbonyls to produce hydrazone, which is analysed with a spectrophotometer (370 nm). The carbonyl content was determined from the difference in absorbance between DNPH-reacted samples and non-reacted HCl samples.

The total protein content was determined in each larval extract with the Coomassie Blue dye-binding method with a standard of bovine plasma albumin (Bio-Rad Protein Assay, Munich, Germany). Because the protein content of larvae changes during the construction of a case in *L. rhombicus* (Mondy et al., 2011) (present study: 30% loss of larval protein content, d.f.=3, F=3.03, P=0.032), the data on total antioxidant activity, SOD activity and protein carbonyl content are reported per mg of total protein mg^{-1} of fresh mass.

Life history traits of adults

The duration of the adult lifetime was recorded under drastic laboratory conditions for 20 newly emerged males and 20 newly emerged females issued from control larvae and from larvae forced to build a new case. Each insect was kept individually in a glass tube containing a small piece of cotton wool saturated with water and renewed daily during the entire lifetime of the insect. Survival was recorded twice a day.

Because many life history and ecological traits are strongly related to body size (Chown and Gaston, 2010; Stillwell et al., 2010), morphological studies were conducted on 20 newly emerged males and 20 newly emerged females issuing from either control or treated larvae. First, the adults were photographed on their left side with a Panasonic Lumix DMC-FS3, and the length of the wing was measured with JMicroVision (V1-2-7 2002–2008, Nicolas Roduit). Second, the adults were lyophilised and weighed. In holometabolous insects, such as Trichoptera, allocation of resources to either adult soma or reproduction occurs during pupation. The amount of resources available for reproduction is approximately equivalent to the amount of resources allocated to the abdomen at emergence (e.g. Stevens et al., 1999; Stevens et al., 2000; Jannot et al., 2007), whereas the amount allocated to soma is equivalent to that allocated to the rest of the body, primarily the thoracic flight muscles (Karlsson and Wickman, 1990). Because reproduction of *L. rhombicus* was impossible in our laboratory, we weighed the thorax and the abdomen separately as indicators of insect dispersion potential and of insect reproductive potential, respectively (Stevens et al., 1999; Stevens et al., 2000; Jannot et al., 2007).

Statistical analyses

The normality of the data was tested with a Shapiro–Wilks test. The control and treated groups at 0 h were checked for the different oxidative balance parameters using Student's test (total antioxidant capacity, t=0.105, P=0.91; SOD, t=0.262, P=0.79; protein carbonyl contents, t=–0.097, P=0.92). Measurements of the antioxidant and oxidative damage markers were analysed with a two-way ANOVA. *Post hoc* comparisons were performed with a Fisher's test. The data on wing size and dry body mass were analysed with an ANCOVA on dry body mass with wing size as a covariate. Previously, we have checked the linear relationship between the variable and the covariable with a generalised linear model (GLM). Adult longevity was analysed with an ANOVA including treatment, sex and their interactions. We used an ANOVA instead of a survival analysis because no individuals were censored and the distribution of longevity was close to normal. However, a survival analysis (Kaplan–Meier) gave similar results (not shown). The computations were performed with R statistical software (R Development Core Team, 2010).

RESULTS

Measurement of larval oxidative stress associated with case construction

A significant increase of TEAC (*ca*. 2-fold) in the *L. rhombicus* larvae was found in the insects forced to build a new case (Table 1, Fig. 1A). A higher TEAC was recorded at 48 and 72 h after the elimination of the original case. However, the TEAC of the treated larvae returned to the same level as the control group 1 week after case elimination (Fig. 1A).

The activity of SOD increased significantly 72 h after the beginning of the reconstruction of a new case (*ca*. 2-fold) (Table 1, Fig. 1B). After 1 week, the results showed a decrease in SOD activity and a level similar to that of the control.

The protein carbonyl content of the larvae forced to build a new case was not significantly different from that of the control larvae (Table 1, Fig. 2).

Study of life history traits of adults

Under our laboratory conditions, no significant difference was observed between the adult lifetime of males and females issued from either the control larvae or the treated larvae (control male: 21.25±7.3 days, control female: 22.9±11.6 days; treated male: 22.2±8.6 days, treated female: 24.5±7.9 days; sex effect F=0.289, P=0.592, treatment effect F=0.005, P=0.942).

The female adults produced from the control larvae and from the larvae forced to build a new case showed a similar body condition, expressed as the relationship between their wing length and their body mass (Fig. 3A, ANCOVA, d.f.=1, F=0.66, P=0.422). In

Table 1. Two-way analysis of variance on larval TEAC, SOD activity and protein carbonyl content with control/treatment and time as factors

		d.f.	F	P
Total antioxidant capacity	Treatment	1	13.61	**<0.001**
	Sample time	2	1.011	0.371
SOD	Treatment	1	4.841	**0.030**
	Sample time	2	1.236	0.301
Protein carbonyl	Treatment	1	2.975	0.090
	Sample time	2	3.031	0.059

TEAC, Trolox equivalent antioxidant capacity; SOD, superoxide dismutase.
For each factor, multiple comparisons were performed with a Fisher's test to describe the differences between groups (see Figs 1, 2).
For each parameter (total antioxidant capacity, SOD and protein carbonyl) the interaction treatment × sample time was not significant, so only the simple effects are presented.
Significant values are in bold.

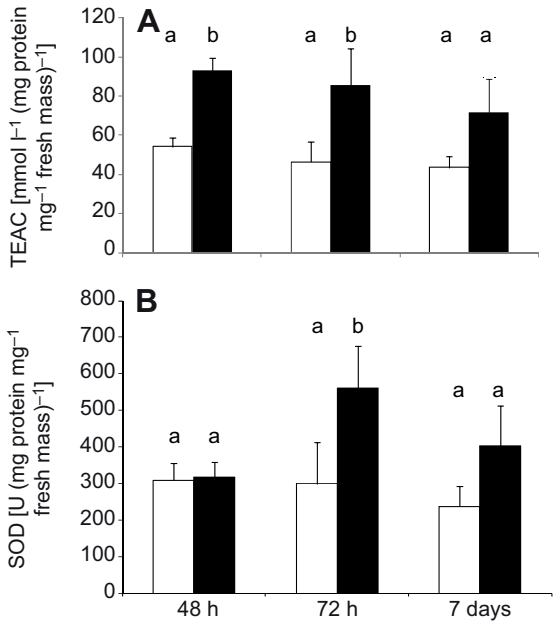

Fig. 1. Oxidative stress defence of control *Limnephilus rhombicus* larvae (open bars) and *L. rhombicus* larvae forced to rebuild a case (filled bars) 48 h, 72 h and 7 days after the elimination of the original case. (A) Trolox equivalent antioxidant capacity (TEAC). (B) Superoxide dismutase activity (SOD). Error bars represent s.e.m. (N=10). Identical letters indicate that no significant difference was found.

contrast, the males issued from the treated larvae showed a lower value of body condition than the adults issued from the control larvae (Fig. 3B, d.f.=1, F=41.51, P<0.001).

The allocation of resources to adult body parts (abdomen and thorax) in males and females expressed as a percentage of the total mass of the thorax and abdomen, did not differ between adults issued from the control larvae and those issued from the larvae forced to rebuild a case (Fig. 4) (d.f.=1, F=2.23, P=0.145).

DISCUSSION

It has been shown that construction behaviour allows organisms to cope with environmental variations to maximise their survival and reproduction and that such behaviour may be mediated by ecological and physiological mechanisms (Hansell, 2005). The results obtained here provide evidence that the increase in metabolic demand associated with case construction triggers ROS production in larval tissues of *L. rhombicus*. Indeed, even though ROS generation was not directly assessed in the present study, the clear induction of antioxidant defences reveals the activation of a protective mechanism to cope with an increase in pro-oxidant molecules. Several other studies, primarily focused on vertebrates, provide indirect support for the hypothesis that the stimulation of aerobic metabolism induces oxidative stress (reviewed in Costantini et al., 2010) (but see Selman et al., 2010). Moreover, based on the variation in antioxidant activities, De Block and Stoks (De Block and Stoks, 2008) suggested that an increased metabolism due to compensatory growth after starvation results in significant ROS production in insect models. In our study, the antioxidant capacity of *L. rhombicus* larvae increased 48 h after the beginning of the experiment, an effect that disappeared after 1 week. Organisms possess a variety of defence mechanisms that can protect them against oxidative stress; these mechanisms are commonly divided into specific and non-specific

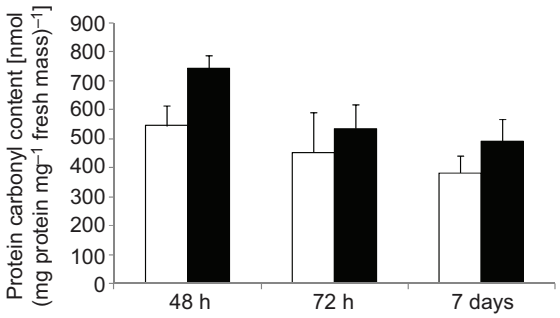

Fig. 2. Protein carbonyl content in control *L. rhombicus* larvae (open bars) and *L. rhombicus* larvae forced to rebuild a case (filled bars) 48 h, 72 h and 7 days after the elimination of the original case. Error bars represent s.e.m. (N=10).

defences. Our results showed a rapid increase of TEAC 48 h after the elimination of the case, whereas SOD activity increased only after 72 h. SOD is a specific antioxidant enzyme that controls the level of intracellular superoxide radicals by dismutating superoxide anions to hydrogen peroxide (reviewed in Dowling and Simmons, 2009) and has been shown to increase during oxidative challenges in insects (e.g. Krishnan and Kodrík, 2006; Mittapalli et al., 2007). The reason that the induction of SOD is delayed relative to the increase in TEAC is not clear. One possibility is that the induction of non-enzymatic antioxidant proteins, such as peroxiredoxin or glutathione (Pamplona and Costantini, 2011), has priority over SOD activation in the early phase of oxidative challenge. Alternatively, the non-protein antioxidant defences of the treated larvae, such as uric acid (reviewed in Costantini, 2008) or dietary antioxidants (reviewed in Catoni et al., 2008), may increase rapidly after the beginning of case reconstruction. An antioxidant role of uric acid has previously been suggested, primarily in birds (reviewed in Costantini, 2008). This compound is also present in insects, where

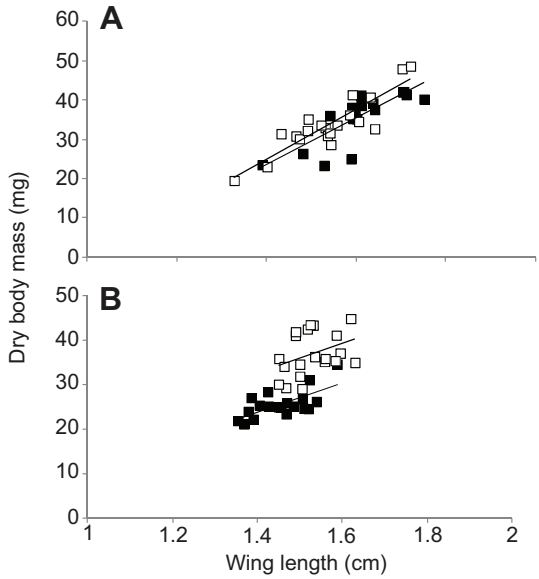

Fig. 3. Linear regression between wing length and dry body mass of (A) *L. rhombicus* females issued from control larvae (open squares, y=63.51x–63.933, R^2=0.77) and from treated larvae (filled squares, y=59.69x–59.95, R^2=0.63) and (B) *L. rhombicus* males issued from control larvae (open squares, y=32.59x–12.77, R^2=0.13) and from treated larvae (filled squares, y=33.33x–22.81, R^2=0.47).

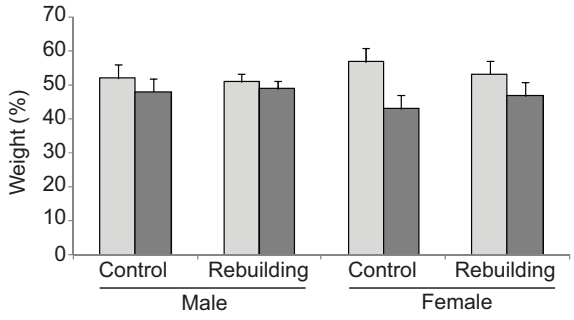

Fig. 4. Thoracic (pale grey) and abdominal (dark grey) dry mass expressed as a percentage of total mass of abdomen and thorax for male and female adults issued from control larvae and from larvae forced to rebuild a case.

it is the principal nitrogen waste product. A second explanation for the lag could relate to the dietary antioxidants, such as vitamin C, vitamin E, carotenoids and polyphenolic antioxidants, which are mainly secondary metabolites that plants synthesise to protect themselves against oxidative stress. Our results suggest that *L. rhombicus* larvae could use these compounds initially before biosynthesising the specific antioxidant enzyme. Indeed, after the elimination of the case, the larvae showed high levels of locomotor activity while searching for and manipulating plants. The *L. rhombicus* larvae cut and collect small pieces of plants for use as building material but could also feed on the same plants. To better understand this outcome, it is important to detect whether larvae show any preference for certain plants during the reconstruction process.

Oxidative stress results from a mismatch between the production of damaging ROS and the organism's capacity to mitigate the damaging effects (Monaghan et al., 2009). In the present study, the protein carbonyl content of the larvae forced to rebuild a case was not different from the protein carbonyl content of the control larvae. Therefore, the antioxidant defences stimulated during the larval construction of a new case gave sufficient protection to the proteins against the harmful effects of ROS. This result confirms our first scenario. As also expected, no significant difference in longevity was recorded between the control adults and the adults issued from the larvae forced to build a new case, but adult body size, particularly in males, was impacted by the larval stress resulting from the reconstruction activity. The morphological parameters (i.e. the dry body mass and wing length) of the adult females were not impacted by the larval reconstruction. In contrast, the adult males produced from the larvae forced to rebuild a case weighed less and were smaller than the control males. However, no association was found between construction behaviour and differential resource allocation to reproduction and dispersal (no relative difference in abdominal or thoracic mass, Fig. 4). This result does not agree with the results of previous studies in other caddisfly species. Indeed, larvae of *Odontocerum albicorne* and *Glyphotaelius pellucidus* forced to rebuild a new case show a differential reduction of their thorax and abdomen sizes (Stevens et al., 2000). Nevertheless, because the formation of testes in *L. rhombicus* larvae occurs at the end of the final larval stage and because gametogenesis is complete at adult emergence (Le Lannic, 1975), the reduction in the abdominal mass of the males produced from the larvae forced to rebuild a case, relative to the control males (loss of 28% in dry mass), could indicate a decrease in their reproductive capacity. Similarly, because in *L. rhombicus* the emerging male can migrate to search for females, the observed thorax reduction (loss of 35% in dry mass) can affect the caddisfly's dispersal ability even if the ratio of thorax to abdomen has been preserved. Because insect reproductive success depends primarily on the searching ability of the male (Rogowitz and Chappell, 2000), such modifications potentially have a strong impact on fitness.

This impact of construction behaviour on morphology could result from differential resource allocation. The maintenance and up-regulation of antioxidant defences and the repair of the damaged molecules require resources that could potentially be allocated to other functions. Nevertheless, the sex-dependent response was intriguing. Combating oxidative stress can be achieved through various alternative physiological adjustments. These adjustments include the reduction of oxidant generation by the mitochondria, the improvement of the resistance of the structural component to oxidation and the stimulation of repair mechanisms and/or of detoxifying antioxidant systems (Pamplona and Costantini, 2011). Variation in the importance of these parameters may explain why the sexes do not respond equally to a perturbation of ROS production or antioxidant resources (Magwere et al., 2006; Monaghan et al., 2009). A recent study showed that female spiders *Brachypelma albopilosa* produced less mitochondrial superoxide than males and are better protected against oxidative attack (Criscuolo et al., 2010). Variability in the internal allocation of metabolites was previously shown in an insect study (Zera and Harshman, 2001) investigating cell fuels that may change the way in which mitochondria function and ultimately drive the different rates of ROS production that have been suggested as a possible explanation of this sex difference (Criscuolo et al., 2010). Uncoupling proteins (UCP) that decrease the mitochondrial transmembrane proton gradient represent another possible explanation of this sex difference. Several studies on uncoupling proteins strongly suggest that these proteins play a role in sex differences in ROS production (Criscuolo et al., 2005; Sureda et al., 2008; Criscuolo et al., 2010). More generally, the stronger effect of stress during early life on the faster-growing sex has previously been suggested (Clutton-Brock et al., 1985; Toïgo et al., 1999; Metcalfe and Monaghan, 2001). The marked protandry often exhibited by insects could partly explain this male susceptibility to early stress. In summary, oxidative stress appears to mediate the cost of construction and impacts life history traits. It thus appears that case construction in *L. rhombicus* requires an efficient antioxidant system probably based on both specific and non-specific defences. However, the consequences of the activation of such cellular protection are sex dependent and differentially affect the morphology of adults. The extent of these effects on behaviours with a high level of energy consumption such as migration or foraging needs further investigation in terms of the sex-specific strategies of oxidative stress management.

ACKNOWLEDGEMENTS

We thank L. Brepson for technical assistance, T. Lengagne, D. Roussel and J. Casas for helpful suggestions, and G. McIlroy for English correction of the manuscript.

FUNDING

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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