Revisiting the link between breeding effort and oxidative balance through field evaluation of two sympatric sibling insect species

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The idea that oxidative stress could be a major force governing evolutionary trade-offs has recently been challenged by experimental approaches in laboratory conditions, triggering extensive debates centered on theoretical and methodological issues. Here, we revisited the link between oxidative stress and reproduction by measuring multiple antioxidant and oxidative damages in wild-caught females of two sibling weevil species (*Curculio elephas*, *C. glandium*). The strength of our study arised from (1) studied species that were sympatric and exploited similar resource, but displayed contrasting reproductive strategies and (2) individuals were sampled throughout adult life so as to relate oxidative status to breeding effort. We found that the short-lived *C. elephas* sacrifices red-ox homeostasis for immediate reproduction upon emergence as characterized by low antioxidant defenses and elevated oxidative damage. Comparatively, *C. glandium* massively invests in antioxidant and maintains low oxidative damage, which may contribute to their extended prereproductive period. Intriguingly, we also reveal, for the first time in a field study, an unexpected reactivation of antioxidant defenses with the onset of reproduction. Our results thus support the existence of a strong, but complex relationship between oxidative stress and life-history evolution and highlight the need for a finer-scale picture of antioxidant strategies.

KEY WORDS: Insect, life-history evolution, oxidative stress, reactive oxygen species, sympatric species, weevil.

The past decade has seen a surge of interest in the role of reactive oxygen species (ROS) and the damage they might cause on biomolecules in mediating life-history evolution (Dowling and Simmons 2009; Metcalfe and Alonso-Alvarez 2010; Selman et al. 2012). ROS are deleterious by-products of aerobic metabolism mainly generated during mitochondrial adenosine triphosphate synthesis (Jezek and Hlavata 2005), yet their harmful effect is buffered by a complex array of antioxidants (Pamplona and Costantini 2011). It is generally accepted that imbalance between ROS production and the efficiency of the cellular antioxidant machinery is likely to cause detrimental accumulation of oxidative damage in tissues, a physiological state referred as "oxidative stress." Oxidative stress plays a crucial role in the etiology of numerous degenerative diseases, it accelerates senescence and affects organismal performance (Finkel and Holbrook 2000). When put into an evolutionary framework, oxidative stress has been considered as a key component underpinning trade-offs between reproduction and somatic maintenance because of positive
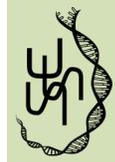
correlation observed between reproductive effort and oxidative stress in mammals (Bergeron et al. 2011; Fletcher et al. 2012), birds (Alonso-Alvarez et al. 2004; Wiersma et al. 2004), and insect species (Archer et al. 2013).

However, the view of oxidative stress as proximal physiological cost of reproduction has recently been challenged by laboratory investigations, showing that oxidative damages are unchanged or even lowered with reproductive effort (Garratt et al. 2011; Oldakowski et al. 2012; Schmidt et al. 2014), and that antioxidant defenses increase during reproductive periods (Garratt et al. 2013; Xu et al. 2013; Michalkova et al. 2014). Such controversial results have been debated because laboratory conditions might not reveal evolutionary trade-offs if animals are provided with nonlimiting resources (Metcalfe and Monaghan 2013). However, the discrepancies between laboratory and field studies may also have other origins, such as the nature of the biomarker measured and of the tissue considered (Speakman and Garratt 2013). For instance, the very large majority of field studies rely on markers measured only in blood, which is a heterogeneous, complex, and versatile tissue. Conversely, laboratory studies clearly indicate that the oxidative response to reproductive effort is tissue-specific and thus question the validity of conclusions drawn only from blood analysis (Garratt et al. 2012; Xu et al. 2013; Schmidt et al. 2014). Discrepancy between laboratory and field studies thus suggests that the relation between reproductive effort and oxidative stress may well be more complex than currently apprehended and highlight the need for complementary studies.

To address the current debate, we investigated the evolutionary link between antioxidant and reproductive strategies in natural populations of insect species. We measured multiple antioxidant defenses (total antioxidant, superoxide dismutase (SOD), and catalase (CAT) activities) and oxidative damage (malondialdehyde [MDA]) in two sibling weevil species (*Curculio elephas* and *C. glandium*). The strength and originality of our study lies in the three following complementary features: our observations allow (1) comparisons of two sibling species facing the same environmental constraints while differing in their reproductive strategies (see below), (2) precise monitoring of the kinetics of oxidative stress markers in parallel with that of reproductive effort in wild-caught insects during their entire adult life span, and (3) assaying oxidative stress markers from the insects' whole body, which overcomes the risk of measuring a tissue-specific biased response to oxidative stress.

The two weevil species compete to lay eggs into oak acorns (*Quercus spp*), whose availability vary greatly, both temporally and spatially (Venner et al. 2011; Pélisson et al. 2013a). The two species evolved markedly distinct strategies: *C. elephas* exhibits variable dormancy duration (ranging from one to four years), so that adults belonging to the same larval cohort emerge over several years. This strategy should ensure that at least some offspring

from a given clutch will encounter locally favorable conditions (i.e., trees with large acorn production) and breed successfully (Venner et al. 2011). *Curculio elephas* is proovigenic, with females emerging late in the season and being short-lived (approximately one month), having mature oocytes upon emergence, and spending most time at laying eggs (Pélisson et al. 2012, 2013a). *Curculio glandium* does not spread the emergence of adults over several years, but copes efficiently with the spatial heterogeneity of the acorn availability (Pélisson et al. 2013b). Adults emerge early in the season, are much longer lived (from April to late September, i.e., six months) than *C. elephas*, and display greater flying capacities (Pélisson et al. 2013b). *Curculio glandium* females are fully synovigenic and start maturing eggs only late in the season.

Considering that the reproduction–survival trade-off might be mediated by oxidative stress, we expect these two species to display contrasted antioxidant strategies. Short-lived *C. elephas* females—displaying fully mature eggs by the time of their emergence—would invest little in somatic maintenance. Accordingly, low level of antioxidant defenses is expected in this species together with high level of oxidative damage. Conversely, adult *C. glandium* females would display efficient antioxidant defense mechanisms by the time of emergence, allowing them to support extended longevity while limiting the accumulation of ROS-related oxidative damage associated with costly flying activity. By the time they start massively investing into reproduction, we expect antioxidant defense level to collapse concomitantly with increasing oxidative damages.

Materials and Methods
SAMPLING NEWLY EMERGED ADULT WEEVILS

We surveyed a community composed of oak weevils located in a fragmented agricultural landscape in France (45° 45′ N; 5° 16′ E). In 2007, we collected infested acorns that had dropped off from the studied trees (*Quercus petraea*). Infested acorns were kept in wire-netting boxes in an outdoor arena allowing us to collect mature weevil larvae on the day of their emergence outside the acorn. Larvae were then placed in containers that had been previously filled with sifted soil and buried under the oak trees (see Venner et al. 2011 for detailed method). The experimental design was left undisturbed for two years. In 2009, we collected *C. glandium* and *C. elephas* at the time of their adult emergence, that is, March and August, respectively (Pélisson et al. 2012).

SAMPLING ADULT WEEVILS THROUGHOUT THE SEASON

In 2009, female weevils were live-trapped at different periods of their adult life (early April up to the end of September). Adult

weevils were collected on a sheet laid under the tree following a standardized branch-beating method (see detailed method in Venner et al. 2011). The collected insects were then kept in a cool box, and brought back to the laboratory on the day of their capture. Species and sex were determined according to morphological criteria (Hoffmann 1954). Only females were kept for our analysis. Insects were weighed using an analytical balance (Scaltec SBA 32, resolution: 0.1 mg) and frozen euthanized at −20°C in individual 2 mL Eppendorf tubes (Sarstedt, Marnay, France) prior to dissection for egg content determination or biochemical analysis (see below).

OOGENESIS AND EGG CONTENT DETERMINATION

To determine whether the dynamic of oxidative stress markers was associated with reproductive effort, we tracked the lifetime dynamics of oogenesis of females of the two species. We dissected females (newly emerged females: nine *C. glandium* and eight *C. elephas*; live trapped females: 34 *C. glandium* and 10 *C. elephas*) under binocular microscope (Zeiss stemi-C; Illkirch, France) and we counted the fully chorioned eggs present in their ovaries.

ANTIOXIDANT DEFENSES

We measured (1) the total antioxidant capacity, resulting from the combined properties of all antioxidant molecules (Trolox equivalent antioxidant capacity [TEAC]) and (2) the specific activity of two inducible antioxidant enzymes: total SOD and CAT enzymes. Antioxidant defenses were assayed on 60 *C. glandium* and 30 *C. elephas* females that had been collected in the field either upon emergence or later at various times of the year.

The dry-frozen insects were individually crushed for 30 sec at 30 Hz in 2 mL Eppendorf tubes containing a stainless steel bead and 350 μL of an extraction buffer (100 mM KH_2PO_4, 1 mM dithiothreitol [DTT], 2 mM ethylene glycol tetraacetic acid [EGTA], pH adjusted to 7.4 at 4°C). All chemicals were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France). Tubes were centrifuged at $10,000 \times g$ for 5 min (4°C) and the supernatant aliquoted and frozen at −80°C until biochemical analysis. Because freezing and vigorous homogenization protocols disrupt cytoplasmic and organelle membranes, our protocol allow determining antioxidant activity of all cell compartments, including mitochondria and peroxisome.

Total tissue antioxidant activity was determined spectrophotometrically at 750 nm (Xenius SAFAS, Monaco) using commercial antioxidant assay kit (Cayman Chemicals, n°709001, Interchim, Montluçon, France). A standard curve was realized using Trolox (antioxidant derivative of vitamin E) and the results were expressed as TEAC. To account for any potential interference between components of the extraction buffer (e.g., EGTA, DTT) and

antioxidant assays, all standard curves were made using extraction buffer.

SOD is a metalloenzyme that catalyses the dismutation of the superoxide anion into molecular oxygen and hydrogen peroxide. Total SOD activity (cytoplasmic and mitochondrial SOD activities) was measured spectrophotometrically (450 nm) using a commercial kit (Cayman Chemical, n°706002).

CAT enzyme catalyses the hydrogen peroxide decomposition to gaseous oxygen and water. CAT activity was determined spectrophotometrically (540 nm) using commercial kit (Cayman Chemicals, n°707002). A standard curve was obtained with known concentrations of formaldehyde. Results are expressed as nanomole formaldehyde produced per minute.

All antioxidant data were corrected per milligram body mass. However, we also verified that our results remain valid when expressed per milligram protein. Because both species display different protein concentration at the time of emergence (Pélisson et al. 2013a), we ran linear model analyses to account for whole body protein concentration (see Supporting Information File). Protein content was quantified spectrophotometrically (595 nm) using Bradford assay (Sigma-Aldrich), following manufacturer's instructions and using bovine serum albumin as standard.

OXIDATIVE DAMAGE DETERMINATION

As an index of oxidative injuries, we determined peroxidized lipid content in homogenates by measuring the level of MDA. Wild-caught females of both species (30 *C. glandium* and 20 *C. elephas*) were individually placed in 2 mL Eppendorf tubes, each containing a stainless steel bead and 200 μL Ripa lysis buffer (50 mM Tris-HCl, 150 mM NaCl, and 0.1% deoxycholate, pH adjusted to 7.4 at 4°C) and crushed 30 sec at 30 Hz (Tissue Lyser, Qiagen). Homogenates were then centrifuged for 10 min at $1600 \times g$ (4°C) and the supernatant stored at −80°C until the assay. MDA was measured fluorometrically following protocols routinely used for estimating oxidative damage to lipids in various species (Rey et al. 2010). A standard was established with known concentration of MDA and the results were corrected per milligram body mass.

STATISTICAL ANALYSIS

We compared the two species for the antioxidant activity and oxidative damages produced by females at two distinct periods, that is, upon emergence and at the time of breeding, using nonparametric Wilcoxon tests (but see Supporting Information Files for further analyses). The seasonal dynamics of antioxidants and of oxidative damages were then analyzed separately for the two species. For the long-lived species (*C. glandium*), data were first analyzed by comparing three periods of life (i.e., at adult emergence, during the prereproductive period, and while breeding), using ANOVA with contrast procedure. Second, we analyzed the changes in oxidative stress markers throughout the reproductive period with

a linear model, including the day of capture within the year (time) and the squared variable (time^2) as covariates, so as to test possible quadratic effect of time. All analyses and figures were performed with the R free software environment (http://cran.at.r-project.org). Data presented in this study are available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.b8nf6.

Results

### OXIDATIVE BALANCE OF C. ELEPHAS AND C. GLANDIUM AT THE TIME OF ADULT EMERGENCE

The level of antioxidant defenses and oxidative damages measured in newly emerged adult females is shown in Figure 1. Newly emerged *C. elephas* females exhibited much less total antioxidant capacity (-40%; $W = 15$, $P < 0.0001$; Fig. 1A) and SOD activity (-49%; $W = 1$, $P < 0.0001$; Fig. 1B) than *C. glandium*, yet no significant difference could be detected in CAT activity between the two species ($W = 130$, $P = 0.48$; Fig. 1C). Similar antioxidant patterns were found when accounting for protein concentration (see Supporting Information File). In terms of oxidative damage, *C. elephas* females showed greater amounts of peroxidized lipids (MDA) than *C. glandium* ($+47\%$; $W = 70$, $P < 0.0001$; Fig. 1D) by the time of their emergence.

### SEASONAL DYNAMICS OF OOGENESIS AND EGG MATURATION

In the short-lived weevil species (*C. elephas*) all the females collected either upon emergence (early August) or collected in the field (August to early September) exhibited mature eggs in their ovaries (Fig. 2A). By contrast, *C. glandium* females showed extended prereproductive period characterized by the absence of eggs in their ovaries from emergence (April–May) up to the end of July (Fig. 2B). These females then entered a two months breeding phase (late July to late September).

ADULT LIFETIME DYNAMICS OF TOTAL AND SPECIFIC ANTIOXIDANT DEFENSES (Fig. 2C–H)

The amount of total antioxidant defenses (TEAC) was significantly lower in *C. elephas* females caught in the field as compared to emerging ones (Fig. 2C, $W = 209$, $P < 0.0001$). However, in *C. glandium*, TEAC remained at a similar level during the prereproductive period as compared with emerging adults (contrast procedure, $t = 1.25$, df $= 57$, $P < 0.21$; Fig. 2D). There was significant quadratic effect of time on TEAC that first increased and peaked during the early phase of the reproductive period before decreasing at the end of the laying period (Fig. 2D, Table 1). When comparing the two species during their reproductive period, TEAC was found significantly lower in *C. elephas* ($W = 0$, $P < 0.001$).
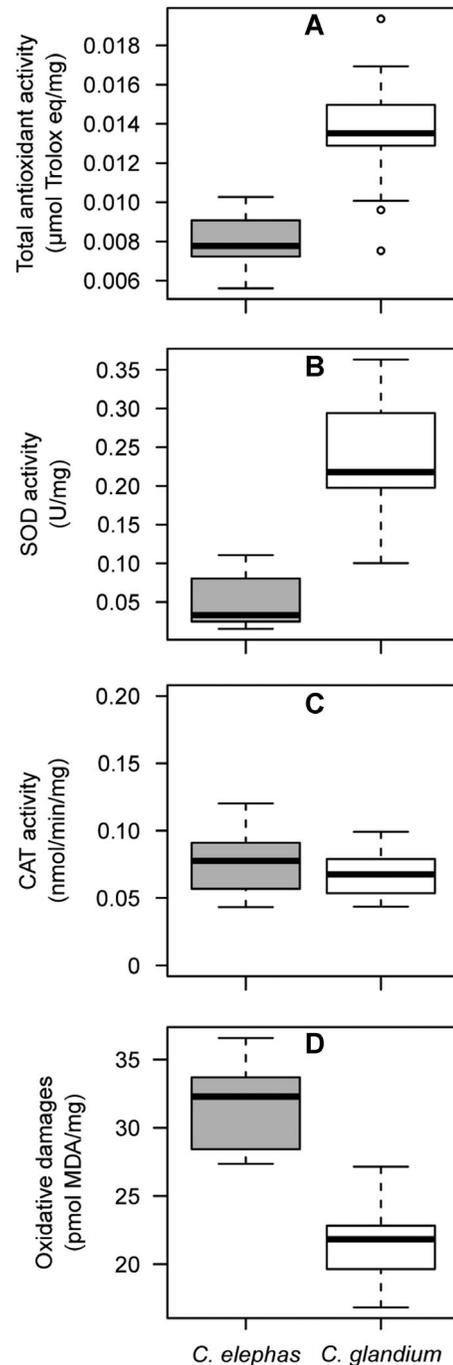
Figure 1. Boxplot representation of antioxidant and oxidative damages to lipids in *Curculio elephas* (gray box) and *C. glandium* (white box) adult females at the time of emergence. *Curculio elephas* is characterized by massive reproduction since the time of their emergence, low dispersal capacities, and short life span. Comparatively, *C. glandium* is long-lived and present a long prereproductive period and high dispersal capabilities. Upper panel shows the total antioxidant defenses (A). Middle panels represent the distribution of specific antioxidant activity of SOD (B) and of CAT (C). Lower panel represents oxidative damage to lipids (D). Boxplots: horizontal bold line, median; box, lower and upper quartiles; dashed lines, 95% confidence.
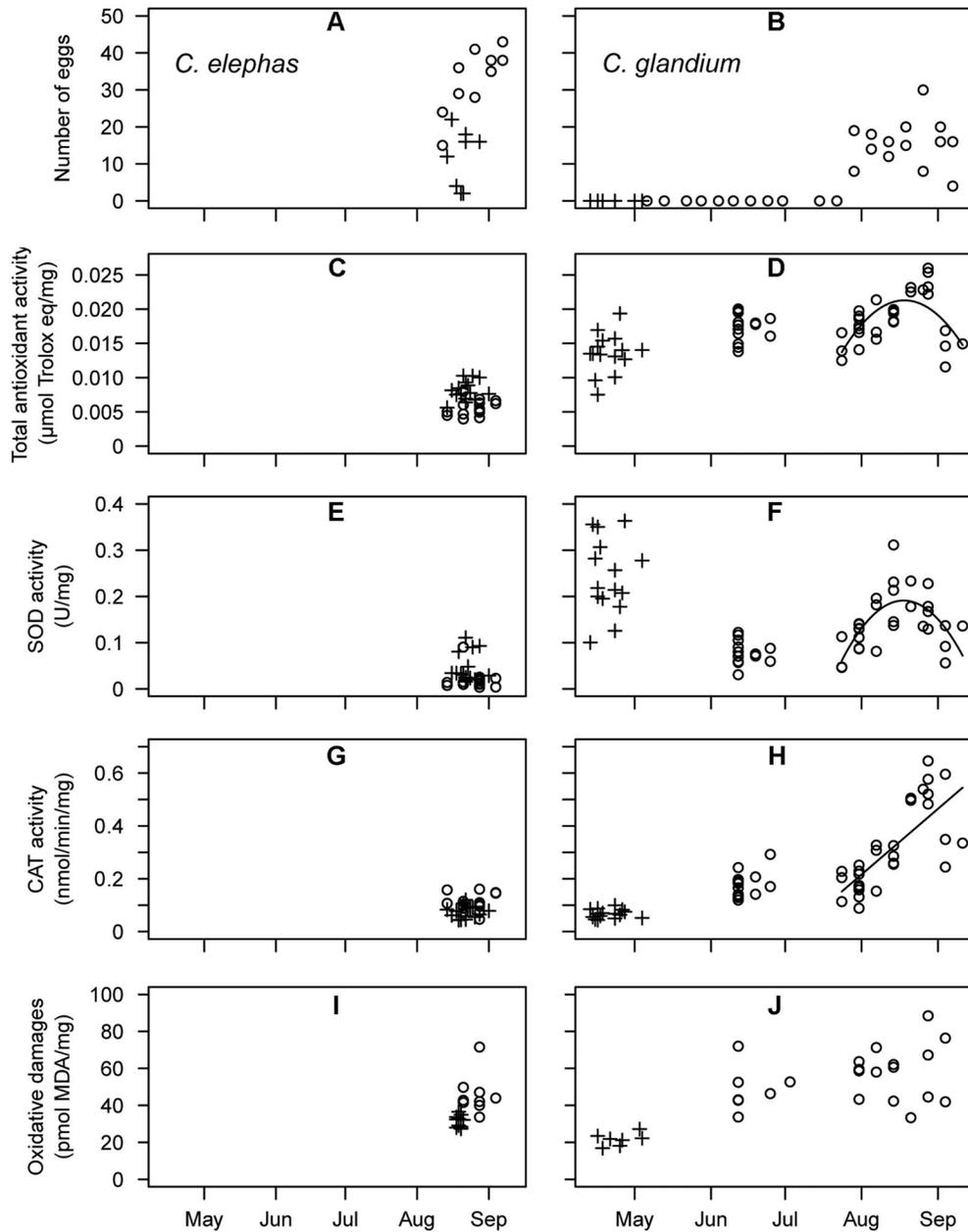
Figure 2. Temporal kinetic in the number of mature eggs found in the ovaries (panels A and B) of the total (panels C and D) and specific antioxidant activity (panels C to H) and of the oxidative damages to lipids (panels I and J) in *Curculio elephas* (left panels) and *C. glandium* (right panels) adult females collected either at the time of their emergence above ground (cross symbols) or in the field at different times of the year (open circles).

The seasonal kinetic of SOD activity in both species was quite similar to that of TEAC. Newly emerged *C. elephas* females showed greater SOD activity than the ones caught later in the field ($W = 188$, $P < 0.0001$; Fig. 2E). *Curculio glandium* also showed greater SOD activity upon emergence than during the prereproductive (contrast procedure, $t = 7.24$, df $= 57$, $P < 0.0001$; a quadratic effect of time was further detected during their reproductive period (Fig. 2F, Table 1). Although breeding,

C. elephas females showed less SOD activity than *C. glandium* ones ($W = 6$, $P < 0.0001$).

The CAT-specific activity departed from SOD and TEAC ones, in that it significantly increased in *C. elephas* females from emergence up to the breeding period ($W = 44$, $P = 0.004$; Fig. 2G). In *C. glandium*, the CAT activity increased during the extended prereproductive period compared to newly emerged females (contrast procedure, $t = 2.6$ df $= 57$, $P = 0.012$). Contrasting with

Table 1. Linear models explaining variation of antioxidant defenses and oxidative stress markers over adult lifetime in *Curculio glandium*.

Marker	Variable	Df	*F*-value	*P*-value
TEAC	Time	1;27	3.71	0.064
	Time^2	1;27	15.87	0.0046
SOD	Time	1;27	3.48	0.072
	Time^2	1;27	19.34	<0.0001
CAT	Time	1;27	36.00	<0.0001
	Time^2	1;27	2.68	0.11
MDA	Time	1;12	0.1003	0.76
	Time^2	1;12	0.1402	0.71

A linear model was fitted on several markers (total antioxidant-TEAC, SOD, and CAT activities; and oxidative damages) measured in *C. glandium* adult females during the reproductive period, by including the time of capture "time" and "time^2" for possible quadratic effect of time as explanatory variables.

both the TEAC and SOD activities in this species, the CAT activity linearly increased during the breeding period (Fig. 2H, Table 1). When comparing both species while breeding, the CAT activity was lower in *C. elephas* than in *C. glandium* females ($W = 23$, $P < 0.0001$).

ADULT LIFETIME DYNAMICS OF OXIDATIVE DAMAGES

In both species, the level of oxidative damages was lower in weevil females that just started their adult life as compared to those caught later in the season (*C. elephas*: $W = 3$, $P = 0.0001$; *C. glandium*: $W = 0$, $P < 0.0001$). In *C. elephas*, although emergence and breeding periods clearly overlapped, adults caught in the field showed higher levels of peroxidized lipids than emerging ones ($+47\%$, $W = 3$, $P = 0.0001$; Fig. 2I). In *C. glandium*, the level of peroxidized lipids first increased during the prereproductive period (contrast procedure, $t = 5.81$, $P < 0.0001$; Fig. 2J) and remained high during the two-month reproductive period (contrast procedure, $t = 1.59$, $P = 0.12$). At the time of breeding, the two species did not statistically differ from each other in their level of oxidative damages ($W = 35$, $P = 0.055$).

Discussion

The relationship between antioxidative defenses and reproductive effort is believed to be a key element for understanding the physiological mechanisms underlying the evolution of life-history traits. However, the exact nature and the extent of such relationship is still under great debate (Monaghan et al. 2009; Oldakowski et al. 2012; Metcalfe and Monaghan 2013; Speakman and Garratt 2013). Here, we address this issue by comparing antioxidative strategies of wild-caught insects belonging to two sibling

sympatric species. These two species face the same environment, yet they display contrasted strategies of energy acquisition and allocation toward reproduction (Pélisson et al. 2012, 2013a).

At first glance, our results support the proposal that investment in antioxidative defenses, linked to reproductive effort, underlies a trade-off between reproduction and somatic maintenance. Compared to *C. glandium*, *C. elephas* adults are short-lived. To compensate for their time-limited reproductive period, *C. elephas* females harbor mature eggs in their ovaries and are ready to mate and oviposit when emerging above ground (Fig. 2A). We show that, at the onset of their adult life, *C. elephas* females are characterized by low levels of total and specific antioxidant defenses (SOD), and high levels of oxidative damages to lipids. In *C. elephas*, the low antioxidant defenses can be interpreted as a strategy in which oxidative balance is sacrificed at the benefit of massive egg production and laying behavior during their short reproductive period. From an evolutionary point of view, this strategy might maximize the potential fecundity of female adults and thus their reproductive success during mast-seeding years, that is, when oak acorn availability is not limiting. In contrast, *C. glandium* females exhibit extended longevity while starting oogenesis only late in the season. Since the time of their emergence, *C. glandium* females invest heavily into somatic maintenance, as characterized by their high levels of total and specific (SOD) antioxidant defenses as well as their low levels of oxidative damage. Furthermore, the long prereproductive stage in *C. glandium* adult females is associated with high dispersal capability by flying as soon as they emerge (Pélisson et al. 2013b). In insects, flying is an expensive behavior known to generate substantial amounts of ROS and consequently ROS-related damage in active tissues, with a direct impact on longevity (Yan and Sohal 2000; Williams et al. 2008). *Curculio glandium* females seem to primarily invest into dispersal and somatic maintenance (i.e., by producing effective antioxidant components) and delay reproductive activity. The antioxidant strategy of *C. glandium* would allow females to live long enough to buffer the spatial fluctuations of the resource by efficiently moving toward trees bearing abundant acorn crops before redirecting their energy into egg maturing and laying. These interspecific differences appear therefore to support the "free radical theory of aging" and the assertion that reproductive effort is provided at the expense of red-ox homeostasis that is likely to accelerate senescence.

However, contrasting with this statement, we also observed in *C. glandium* females an unexpected transient reactivation of their total antioxidant defenses and in their SOD activity (Fig. 2D, f), together with a linear and continuous increase in their CAT activity (Fig. 2H) throughout their extended breeding period. Furthermore, there was no clear evidence of any increased oxidative damage when shifting from the prereproductive to the reproductive period. These results do not match the classical hypothesis of a

trade-off between reproduction and red-ox homeostasis (Wiersma et al. 2004; Metcalfe and Alonso-Alvarez 2010). On the contrary, they show some similarities with recent controversial empirical findings in rodents (Garratt et al. 2011; Xu et al. 2013; Schmidt et al. 2014) and insects (Michalkova et al. 2014) held in standardized laboratory conditions.

All together, our results support the existence of a strong, yet complex, relationship between oxidative stress and reproduction. We suggest that the stimulation of antioxidant defenses may play a key role in preserving the red-ox homeostasis and genome integrity of germ cells during the critical phase of oogenesis. Accordingly, the specific increase in CAT activity observed during the breeding period may be linked to the need for fatty acids to be mobilized for vitellogenesis. CAT is highly concentrated in the peroxisome, an organelle which is involved in lipid metabolism, and which produces considerable amounts of hydrogen peroxide. Activating peroxisomal CAT may protect organisms against the oxidative stress associated with lipid metabolism during reproductive effort (Bonekamp et al. 2009; Speijer 2010). Previous laboratory studies in rodents have shown that antioxidant defense, including SOD and CAT, are upregulated during reproductive periods (Garratt et al. 2013). It has been suggested, however, that animals are unable to upregulate antioxidant defense while reproducing in the field, because they have limited resources (Metcalfe and Monaghan 2013). Our study gives the first counterexample in a natural population of antioxidant defenses increasing along reproductive effort.

The complexity of the relationship between antioxidants and reproductive effort highlight the need for a finer-scale picture of antioxidant strategies. For that purpose, combining multiple assays and taking advantage of high-throughput sequencing fitted to nonmodel species (Dégletagne et al. 2010) might help exploring complementary aspects of the oxidative balance, such as the efficiency of the DNA repair mechanism, or the intrinsic susceptibility of biomolecules to ROS (Rey et al. 2014). In complement, studying mitochondrial plasticity regarding ROS generation versus their ability to supply ATP remains to be investigated in the context of life-history evolution (Salin et al. 2012a, b; Pichaud et al. 2013). Such physiological mechanisms may constitute key levers for controlling red-ox homeostasis and clearly deserve further investigations to better understand life-history trait evolution.

As a perspective in community ecology, our results also suggest that diversification of antioxidative strategies between competing species may contribute to the evolution of contrasted physiological, behavioral, and life-history traits, and thus constitutes a key component of their ecological niche partitioning and coexistence. The two sibling weevil species under study coexist while competing for egg laying into the same resource (oak acorns). They exhibit sharply contrasting antioxidative strategies that might promote their differentiation in resource acquisition and allocation strategies, and consequently their spatial and temporal partitioning of resource exploitation that is central for stabilizing their coexistence (Venner et al. 2011; Pélisson et al. 2012, 2013b). However, due to the correlative nature of our study, the causative link between antioxidative strategies and ecological niche partitioning could not be firmly established. Indeed, we can hypothesize that competition between species constitutes a selective pressure that might favor the divergence of antioxidative strategies, which would be the first step toward niche partitioning among species. Alternatively, competition could have directly promoted the divergence of phenotypic traits, leading to ecological niche partitioning, prior to the diversification of antioxidative strategies that could have subsequently helped each weevil species optimizing the use of its new ecological niche. Nevertheless, our data provide the first evidence of a link between antioxidative strategy diversification and ecological niche partitioning between competing species opening new research perspectives at the crossing point between oxidative stress physiology and community ecology.

DATA ARCHIVING
The doi for our data is 10.5061/dryad.b8nf6/1.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. This figure provides further evidence of intrinsic differences occurring between newly emerged females of *Curculio elephas* and *C. glandium* in terms of antioxidant defense investment (see Fig. 1).