Social factors have important effects on individuals’ fitness, yet the proximate mechanisms remain virtually unknown. Oxidative stress, which results from the imbalance between the systemic effect of reactive oxygen species on molecules and an organism’s capacity to restore oxidative balance, is one potential mechanism mediating such effects. Although individuals’ oxidative balance seems to be affected by their social status, the effects of social environment, and particularly group size and composition, on oxidative balance have seldom been determined in wild populations. Here, we investigated the influence of group size and composition on the oxidative balance of wild dominant Alpine marmots (Marmota marmota). We simultaneously measured an index of circulating oxidative stress (thiobarbituric acid reactive substances) and specific activity of the main enzymatic antioxidant defense (superoxide dismutase). Our results showed a marked decrease in both oxidative damage and antioxidant activity in individuals from larger groups, highlighting a beneficial effect of group size on individuals’ oxidative balance. We discussed the possibility that these physiological benefits originate from social thermoregulation alleviating the cost of hibernation. This study extends our understanding of the proximate factors underpinning the evolution of sociality and opens up novel perspectives within the field of evolutionary ecology.

Key words: antioxidant, group size, marmot, oxidative stress, sociality.

INTRODUCTION

In social species, the characteristics of the group an individual lives in, such as size and composition, can have a considerable impact on its fitness (Krause and Ruxton 2002). For instance, fecundity increases with group size in meerkats (Suricata suricatta, Hodge et al. 2008), whereas offspring survival and reproductive lifespan decrease with the number of same-sex subordinates in the group in prairie voles (Microtus ochrogaster, Mguire et al. 2002) and Alpine marmots (Marmota marmota, Lardy et al. 2015). So far, although the adaptive value of various group characteristics have received great attention (Silk 2007; Ebensperger et al. 2012), the proximate mechanisms mediating such effects are still poorly known (Gredel et al. 2013).

Because oxidative stress is influenced by environmental conditions (Pascual et al. 2003; Bergeron et al. 2011) and may impact individual performance (Bize et al. 2008; Monaghan et al. 2009; Salin et al. 2012), it appears a likely physiological mechanism underlying the effects of social environment on fitness. Oxidative stress is a physiological state resulting from the detrimental accumulation of oxidized molecules in tissues [hereafter referred as oxidative damage]. Oxidative stress occurs when the generation of reactive oxygen species (ROS) overcomes the organism’s detoxifying antioxidant mechanisms may come at a cost to investment in reproduction and/or growth. Second, if antioxidants cannot fully neutralize the damaging effects of excess ROS, the accumulation of oxidized molecules in tissues may impact individuals’ performance and accelerate senescence (reviewed in Monaghan et al. 2009).

Social conditions may influence individual ROS production and oxidative status in different ways. Isolation, crowding, and confrontation have been experimentally shown to cause oxidative stress in laboratory mice (Miyashitaa et al. 2006). In field studies, social environment is also known to influence many aspects of
individuals’ physiology (Creel et al. 2013), notably by modulating the hypothalamic–pituitary–adrenal axis activity (Creel 2001). For instance, social status (reviewed in Creel 2001), group size (e.g., ring-tailed lemurs [Lemur catta], Pride 2005), and group sex-composition (e.g., Assamese macaques [Macaca assamensis], Furtbauer et al. 2014) are all social factors leading to variations in glucocorticoid levels. Such alteration of the endocrine landscape frequently results in oxidative stress (Kotschal et al. 2007; Costantini et al. 2011). On the other hand, group living can modulate an individual’s access to resources (food or reproduction), and both food availability (Pascal et al. 2003) and reproductive status (Cram et al. 2014) are known to influence individual variation in oxidative status. In fact, social status has been shown to affect oxidative stress markers in various species (van de Crommenacker et al. 2011; Beaulieu et al. 2014; Cram et al. 2014; Lewin et al. 2015). Overall, dominants exhibit higher levels of plasma oxidative damage than subordinates during or after the reproductive season, suggesting that oxidative balance of dominants is influenced by reproductive effort that subordinates do not have to endure (van de Crommenacker et al. 2011; Beaulieu et al. 2014; Cram et al. 2014). Like social status, group size, and composition can also modulate reproductive costs (Cram et al. 2015) and more generally alter the physiological state of individuals living in social groups (reviewed in Creel et al. 2013). Nonetheless, the effect of group characteristics on individuals’ oxidative status remains poorly known.

In the present study, we tested the link between 2 key group characteristics (size and composition) and oxidative status in dominant male and female Alpine marmots (Marmota marmota). Alpine marmots are cooperatively breeding rodents, which live in family groups exhibiting strong variation in their size, ranging from 2 to 16 individuals per group. Reproduction is entirely skewed toward the dominant pair of each family group, and thus reproductive costs and intrasexual competition for dominance are constraints unique to these individuals. Previous studies in this species have showed that dominants’ fitness depends on group size and other group characteristics (Lardy et al. 2015). Dominants accumulate both benefits and costs from living in groups, and an optimal group size and composition were previously shown (Lardy et al. 2015). Indeed, the average group size over a dominant lifetime is positively linked to its fitness, but only up to an optimal average size of 6 individuals for males (Lardy et al. 2015). Accordingly, we expected oxidative damage to decrease with group size in males and females, at least until a critical group size. Moreover, dominants living with a large number of same-sex subordinates display lower body mass (Lardy et al. 2012, 2013) as well as lower fitness (Lardy et al. 2015) than those individuals living with fewer same-sex subordinates. This underlines that increased intrasexual competition has important consequences for dominants’ performance in Alpine marmots. Given such a strong intrasexual competition, we also expected sex-specific relationships between group composition and individual oxidative status. Particularly, we expected oxidative damage to rise as the number of same-sex subordinates in the group increased. We also measured enzymatic antioxidants activity in order to decipher between alternative scenarios (Costantini and Verhulst 2009). According to our hypothesis, lower oxidative damage is expected in dominants of larger groups than in those from smaller groups. A high enzymatic antioxidant activity in these individuals may therefore reflect compensatory responses for elevated ROS generation. Alternatively, low oxidative damage concomitant with low enzymatic antioxidant activity may reveal lower ROS generation in large groups than in small groups.

**MATERIAL AND METHODS**

**Study organism**

The Alpine marmot is a hibernating ground-dwelling squirrel, living in family groups of 2–16 individuals (median = 4), composed of a dominant pair, sexually mature (2 years and older) and immature (younger than 2 years old) subordinates of both sexes (number of subordinate males: median = 1 [0–5]; number of subordinate females: median = 1 [0–4]), and pups born that year (Allainé 2000). Alpine marmots are territorial, and the territory, shared by all members of a family, is mainly defended by the dominant pair.

Dominants mate during the 15 days following emergence from hibernation (i.e., from early to late April). After 30 days of gestation, dominant females give birth to the sole litter of the year (1–7 pups, median = 4). The altricial offspring stay in the natal burrow during 40 days and once weaned emerge above ground between mid-June and mid-July. Dominants inhibit reproduction of same-sex subordinates through aggression (Arnold and Dittami 1997; Hacklander 2003), resulting in a high level of corticosteroids that limits testes maturation and spermatogenesis in subordinate males (Arnold and Dittami 1997) and that leads to the failure of embryo implantation and development in subordinate females (Hacklander 2003). Previous work has suggested that the control of many subordinates is energetically costly for dominant males. Consequently, dominant males are more likely to be cuckolded and/or to lose their dominant position when group size increases (Lardy et al. 2012).

Subordinate males are considered to act as helpers. When group members hibernate together from mid-October to early April, subordinate males actively participate in heat production, a phenomenon called social thermoregulation (Arnold 1988). Hibernation is characterized by a cyclic process with alternate hypothermia and euthermia phases (Arnold 1990). At each cycle, subordinate males wake up earlier and have longer euthermic periods than other family members, thus warming the burrow. Consequently, the presence of subordinate males in a family group increases the probability that offspring survive their first hibernation (Allainé and Theurie 2004). Similarly, dominant pairs hibernating alone have a lower chance of survival than dominant pairs hibernating with subordinates (Arnold 1990).

**Field methods**

Data were collected between April and July from 2009 to 2011 in a wild population of Alpine marmots located in the Nature Reserve of La Grande Sassière (2340 m a.s.l., French Alps, 45°29′N, 65°90′E) followed for 25 years. Every year since 1990, marmots belonging to 25 family groups have been captured from mid-April to mid-July using 2-door live traps baited with dandelions (Taraxacum densiflorus) and placed near the entrances of the main burrows in order to assign each captured individual to its family group.

Once captured, individuals were anaesthetized with Zoletil 100 (0.1 mL/kg), sexed, aged, and their social status was determined according to scrotal development for males and teat development for females. All individuals were marked using a transponder (Trovan™, Germany) and a numbered metal ear tag placed on the right ear of females and on the left ear of males. An additional colored plastic ear tag was placed on the opposite ear of dominant individuals.

**Description of family groups**

The composition of family groups was assessed from both capture-recapture data and intensive observations. The number of
subordinates of each sex and age class (pup, yearling, 2 year old, and adult) was assessed for each family group, and scent marking behavior was used to confirm the identity of the dominant pair (Bel et al. 1995).

Each group was then characterized by its size, defined as the annual number of individuals of 1 year old and older in the group (including the dominant pair) and by its composition, defined by the annual number of sexually immature and mature subordinate males and females (males or females aged of 1 year or more).

### Biochemical assays

Between 2009 and 2011, a total of 115 blood samples (5 mL) were drawn from the great saphenous vein of 67 dominant individuals (34 females and 33 males). Blood samples were transferred into heparinized tubes and centrifuged for 5 min at 5000 × g (4 °C). Hemolyzed plasma samples were discarded (N = 11). Plasma samples were aliquoted and stored at −20 °C until analyses.

To assess the level of oxidative damage, we assayed plasma thiobarbituric acid reactive substances (TBARS) as an index of lipid peroxidation. TBARS are natural products of lipid peroxidation commonly used to determine the degree of oxidative stress in various taxa, in physiology (Salin et al. 2012; Rey et al. 2014), ecology, and evolutionary studies (Nussey et al. 2009; Bergeron et al. 2011; Rubolini et al. 2012). TBARS levels were determined spectrophotometrically using a commercial determination kit (TBARS Assay Kit No. 10009055; Cayman Chemical, Ann Arbor, MI) using a standard curve with known amount of malondialdehyde. Intraclass correlation coefficients (ICC), calculated using a 2-way mixed effect ICC with an absolute agreement definition of concordance, were used to test for the consistency of 134 duplicated samples of plasma of Alpine marmots (ICC: r = 0.98, P < 0.001). This method was employed for all ICCs used in our study, as it assesses not only the correlation but also the exact similitude between replicated assays. TBARS levels were measured in 64 individual in total, for 1 year in 33 individuals, for 2 years in 22 individuals, and for 3 years in 9 individuals.

We determined the antioxidant capacity of plasma by quantifying the specific activity of the superoxide dismutase (SOD) using commercial kits (Superoxide Dismutase Assay Kit No. 706002; Cayman Chemical, Ann Arbor, MI) according to the manufacturer’s instructions. By catalyzing the dismutation of the superoxide radicals into oxygen and hydrogen peroxide, SOD is the first line of enzymatic antioxidant defenses playing a key role in redox homeostasis. The assay quantifies the capacity of SOD present in the samples to counteract superoxide radicals generated in vitro by the xanthine oxidase–hypoxanthine system. Free radicals escaping from SOD activity react with the tetrazolium salt allowing spectrophotometric detection at 450 nm against standard curve of known amount of SOD. The consistency was calculated for a subset of 153 duplicated plasma samples of Alpine marmots (ICC: r = 0.99, P < 0.001). SOD activity was measured in 65 individual in total, for 1 year in 35 individuals, for 2 years in 21 individuals, and for 3 years in 9 individuals.

### Statistical analyses

We log-transformed the levels of TBARS and SOD activity to meet the assumption of normality regarding the distribution of the residuals of the following models. We considered TBARS levels and SOD activity of dominant individuals as dependent variables in separate linear mixed models. To test for a link between group size and TBARS levels and SOD activity, group size (range: 2–11) was included as a main effect in the 2 models. We tested for both a linear and a quadratic effect of group size. To control for potential confounding effects, the following explanator variables were included in models: Julian date of capture (range: 22 April–2 July); year of capture (3-level factor: 2009, 2010, 2011); individuals’ age (range: 3–12 years), sex, and body mass (range: 2950–4950 g); litter size for the current breeding season (range: 0–6 pups); and territory quality (assessed by territory aspect: north-facing vs. south-facing slope). Although inspection of the residuals from models including only simple effects did not show any evidence of potential interaction between the considered explanatory variables, biologically meaningful 2-way interactions, such as between group size and the sex, the age of the dominant individual and the litter size as well as between litter size and the sex, were tested and removed if not significant following a backward procedure. We further included the identity of individuals as a random intercept effect to account for repeated measures on the same individual. The territory was not included as a random intercept in the model as dominant individuals always stayed on the same territory and no more than a single dominant occupied a given territory over the entire course of this study.

Individual repeatability for both TBARS levels and SOD activity between 2 events of capture was estimated as the ratio of the variance associated with the individual identity effect divided by the total phenotypic variance (i.e., sum of individual and residual variances). We also tested whether individuals that had high levels of TBARS across the different sampling events showed high SOD activity by calculating covariances across the response variables on the different grouping levels using a bivariate linear mixed model (Dingemans and Dochterman 2013). Both TBARS levels and SOD activity were included as dependent variables, and all significant explanatory variables were retained in the final models for both TBARS and SOD.

Additionally, to test for a sex-specific effect of group composition on oxidative status, we built models including the number of same-sex subordinates (range: 0–5) and the number of other-sex subordinates (range: 0–5) as explanatory variables instead of group size. The effects of group size and group composition were tested in separated models because group size was highly correlated with the number of same-sex (in males: r = 0.89, N = 54, P < 0.001, in females: r = 0.86, N = 61, P < 0.001) and opposite-sex subordinates (in males: r = 0.88, N = 54, P < 0.001, in females: r = 0.88, N = 54, P < 0.001). The number of same-sex subordinates and of opposite-sex subordinates were also correlated, but to a lesser extent (in males: r = 0.54, N = 54, P < 0.001; in females: r = 0.53, N = 61, P < 0.001). To control for potential colinearity, we calculated the variance inflation factor (VIF) for each model. As recommended by Nakazawa (2011), all the VIF values of our models were <2. We included the same confounding variables and followed the same procedure as described above. Models were fitted with the function lme of the nlme package (R package version 3.1-98, Pinheiro et al. 2011) in R (version 2.12.0, R Development Core Team 2010).

### RESULTS

Oxidative damage in dominant individuals, assessed by the levels of circulating TBARS, decreased significantly with group size (β = −0.07 ± 0.03, t = −2.40, P = 0.02, N = 104 observations on 64 individuals, Table 1, Figure 1). TBARS levels varied between years, increased with age of the dominant, and tended to increase with
### Table 1

Effects of group size and confounding variables on plasma oxidative damage (assessed by level of TBARS) of dominant Alpine marmots (n = 104 observations on 64 individuals)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Estimates ± standard error</th>
<th>t value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>3.11 ± 0.59</td>
<td>5.28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Group size</td>
<td>−0.07 ± 0.03</td>
<td>−2.40</td>
<td>0.02</td>
</tr>
<tr>
<td>Year (2010)</td>
<td>0.32 ± 0.13</td>
<td>2.40</td>
<td>0.02</td>
</tr>
<tr>
<td>Year (2011)</td>
<td>−0.30 ± 0.15</td>
<td>−2.04</td>
<td>0.05</td>
</tr>
<tr>
<td>Age</td>
<td>0.06 ± 0.03</td>
<td>2.11</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Nonsignificant terms

| Litter size × sex (male)       | −0.11 ± 0.06              | −1.91   | 0.06    |
| Litter size                   | 0.05 ± 0.04               | 1.14    | 0.26    |
| Sex (male)                    | 0.001 ± 0.175             | 0.01    | 0.99    |
| Territory aspect              | −0.14 ± 0.11              | −1.29   | 0.20    |
| Body mass                     | 0.0002 ± 0.0001           | 1.26    | 0.21    |
| Date of capture               | −0.004 ± 0.004            | −1.00   | 0.32    |
| Group size^2                  | −0.004 ± 0.008            | −0.42   | 0.67    |
| Group size × sex (male)       | 0.03 ± 0.05               | 0.69    | 0.49    |
| Litter size × age             | −0.005 ± 0.01             | −0.39   | 0.70    |

Bold lines indicate statistically significant effects at α = 0.05.

Litter size in females (Table 1). TBARS levels did not depend on territory quality, date of capture, or dominant body mass (Table 1). As with group size, oxidative damage tended to decrease with the number of opposite-sex subordinates ($\beta = −0.09 ± 0.05, t = −1.91, P = 0.06, N = 104 observations on 64 individuals$) but was not influenced by the number of same-sex subordinates ($\beta = −0.05 ± 0.05, t = −1.00, P = 0.32, N = 104 observations on 64 individuals$).

Individual repeatability between annual capture events was low ($r = 0.003, within-individual variance = 0.27, between-individual variance = 0.005$) suggesting no consistency in individual TBARS levels.

Plasma antioxidant defense, assessed by SOD activity, also decreased with group size ($\beta = −0.15 ± 0.03, t = −4.26, P = 0.002, N = 104 observations on 65 individuals$; Table 2, Figure 2). SOD activity was not influenced by any of the confounding variables considered (Table 2). As for group size, antioxidant defense decreased with the number of opposite-sex subordinates ($\beta = −0.20 ± 0.06, t = 3.38, P = 0.002, N = 104 observations on 65 individuals$) but was not influenced by the number of same-sex subordinates ($\beta = −0.08 ± 0.06, t = −1.30, P = 0.20, N = 104 observations on 65 individuals$). Individual repeatability of SOD activity between 2 events of capture was also low ($r = 0.07, within-individual variance = 0.38, between-individual variance = 0.03$). Testing the effect of group size on TBARS levels and SOD activity simultaneously in the bivariate model revealed that covariance between TBARS and SOD on the random levels was positive ($r = 0.10$), indicating that the years individuals had a high level of TBARS they also had a high SOD activity.

### DISCUSSION

The potential effects of social factors on oxidative stress have only recently received attention (van de Crommenacker et al. 2011; Beaulieu et al. 2014; Cram et al. 2014), and the link between group characteristics and oxidative status has never been tested in social mammals. In this study, we showed a negative relationship between group size and circulating TBARS levels, a proxy of oxidative damage, in a wild cooperatively breeding mammal. Low levels of TBARS were associated with low levels of SOD activity, a marker of the antioxidant defense, in dominants when group size increased. This indicates that dominant marmots of large groups accumulate oxidative damage at a lower rate compared with those of smaller groups and this is unlikely due to a higher investment in antioxidant system. In addition to the low individual heterogeneity observed in TBARS and SOD activity levels, our results suggest that dominant marmots of larger groups are not of better quality but rather individuals generating lower oxidative molecules, probably through beneficial effects associated with large group size. Further investigation would be needed to confirm this mechanism. Although this conclusion derives from proxies of oxidative stress measured only in plasma, which may not necessarily reflect the global physiological response (Veski et al. 2009), our data suggest a beneficial effect of group size on oxidative status in marmots.

To date, only few studies have investigated variation in oxidative stress between individuals in social species. All these studies showed differences in oxidative stress across dominance status.
In Alpine marmots, benefits of group living could mostly arise from social thermoregulation, that is, hibernation of several individuals in the same burrow (Armitage 2007). During hibernation, marmots lose on average 30% of their body mass, but this loss is strongly reduced in large groups (Arnold 1990). As hibernation induces extensive release of oxidized molecules, which destabilizes the oxidative balance (Osborne and Hashimoto 2006; Orr et al. 2009), it is likely that higher number of individuals in the burrow alleviates the costs of hibernation and in turn dominant individuals belonging to larger groups would lower oxidative stress. It is also possible that the presence of helpers in the burrow during hibernation decreases some costs of reproduction. Previous studies in this species showed that adults lose more weight during hibernation when pups are present in the burrow but to a lesser degree in the presence of helpers. If helpers can contribute to social thermoregulation and warm offspring up, it would save energy for the parents and thus reduce the costs of reproduction, although this requires further study.

During the activity period, between April and October, individuals in large groups may also have a relaxed physiological stress through, for instance, reduced vigilance behavior and more time available for feeding. In the yellow-bellied marmot (Marmota flaviventris), group size is negatively linked to vigilance (Carey and Moore 1986), though this has not been quantified in Alpine marmots. Finally, the observed decrease in oxidative damage with group size could also arise from a positive correlation between territory quality and the size of the group. Nonetheless, our data do not support this idea as territory aspect, the more relevant factor that may influence territory quality, did not impact dominant oxidative stress. Moreover, over the 25 years of monitoring, the numbers of individuals present in a territory have strongly varied from one year to another, and none of the territories consistently hold more individuals than others (Lardy S, Cohas A, unpublished data). Finally, given that dominant marmots occupy the same territory for life and that the territory surface remains constant whatever the size of the group, one would expect oxidative damage to increase with group size due to intragroup resource competition, contrary to our results. Territory quality is thus unlikely to explain the observed variation in oxidative status.

Contrary to our expectations, we did not find a negative relationship between the number of subordinates of the same sex of the dominant and its oxidative status. Instead, we observed the same pattern for the number of opposite-sex subordinates as for group size, suggesting that the effect of the number of male or female subordinates is due to the correlation between group size and composition rather than to any sex-specific effect of the number of subordinates on the dominants’ oxidative status. We also found that the age and the sex of the dominant tended to influence the level of oxidative damage. Increased oxidative damage with age has been shown in other wild mammals (see for instance in chipmunks [Tamias striatus], Bergeron et al. 2011, or, in mandrills [Mandrillus sphinx], Beaulieu et al. 2014), suggesting that old individuals are not able to counteract accumulation of oxidative damage, which may accelerate somatic senescence. Sex-specific oxidative stress level has been also previously reported and may result from sex-specific physiological needs and/or differential investment in reproduction (Alonso-Alvarez et al. 2004; Rubolini et al. 2012). Here, blood sampling occurred between early May and mid-June, a period during which females are either pregnant or lactating. This may explain the tendency of increased oxidative damage with litter size observed in females. Nonetheless, the size of the group did not influence the link between litter size and oxidative stress suggesting no modulation of females’ reproductive investment by the number of subordinates in the group.

Future studies investigating how social factors impact the physiology of dominant individuals will help us to decipher the proximate mechanisms underlying the evolution of sociality. We particularly encourage studies aiming to understand how group size modulates the physiological costs endured by dominants at different life-history stages.

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