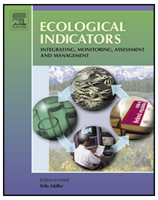
Ecological Indicators 72 (2017) 444–451

Contents lists available at ScienceDirect

Ecological Indicators

journal homepage: www.elsevier.com/locate/ecolind



Original Articles

Wood ant colouration as an ecological indicator for the level of disturbance in managed coniferous forests

Oksana Skaldina*, Jouni Sorvari

Department of Environmental and Biological Sciences, University of Eastern Finland, P.O. Box 1627, FI-70211 Kuopio, Finland

ARTICLE INFO

Article history:
Received 23 October 2015
Received in revised form 2 August 2016
Accepted 18 August 2016

Keywords:
Clear-cutting
Coloration variability
Fluctuating asymmetry
Formica aquilonia
Melanisation

ABSTRACT

The search for ecological indicators of population well-being in natural and managed ecosystems is a crucial aspect of effective biomonitoring, conservation and nature protection. In long-term monitoring programs environmental stress has a measurable effect on naturally selected traits, such as body shape or size. However, changes in colouration provide information about early warning responses. The red wood ant *Formica aquilonia* is ecologically and territorially dominant among wood ant species in European boreal coniferous forest, and possesses variable red-brownish melanin-based cuticular colouration. *F. aquilonia* gynes, which are unfertilized queens, exhibit colour variability on the head, propodeum and abdomen, yet only head colour features allow setting clearly visible and symmetrical (left/right) classes of morphs. We studied phenotypic colour variability, melanisation and fluctuating asymmetry of colour patterns in faces of *F. aquilonia* gynes from natural (forest interiors) and disturbed (clear-cut zones and forest edges) habitats in Finland. We defined five variable, clearly visible and symmetrical (left/right) colour morphs of gynes' faces. Individuals of totally dark morph were present only in disturbed habitats. General analyses of melanisation degree showed, that gynes from disturbed habitats were significantly darker compared to those from forest interiors. Individuals from the same nest tend to have a similar degree of melanisation. The mean darkness of face was highest among individuals from forest-edge nests and the lowest from forest interior nests. In all habitat types the darkness of face increased with an increase in head width. Increase of cuticular melanisation in *F. aquilonia* gynes in disturbed habitats could be explained with the theory of thermal melanism and stress-induced immune defence. Although some amount of fluctuating asymmetry among left/right symmetrical colour variations on faces of *F. aquilonia* gynes was found, it was affected neither by habitat type nor by head width. Melanisation degree of red wood ants have a potency to be used as ecological indicator for the level of disturbance in managed coniferous forests and fluctuating asymmetry of colour variations in ants might be studied furtherly in cases of more severe environmental concerns.

1. Introduction

Habitat destruction is the leading cause of species extinction and the main "biodiversity hotspot" habitats are already disproportionately reduced (Pimm and Raven, 2000). The biggest causes of habitat destruction are: agriculture, mining, trawling, urban sprawl and clear-cut logging. In boreal forests, habitat loss has historically been low and these habitats have been less severely reduced than others, e.g., Mediterranean, broadleaf, mixed, tropical and subtropical forests. Nevertheless, due to active forest management for timber and pulp production, coniferous boreal forests have largely converted from old-growth to timber-production forests with simplified stand structure and species composition (Laurance, 2010). Clear-cutting causes multiple and variable effects on animal communities and different taxa respond to it in various ways. In comparison between the degree of tree removal (clear-cutting, seed tree, shelterwood, patch cutting, group and single tree selection) and various types of natural disturbance (landslide, wildfire, insects, wind, disease) clear-cutting is equal to the most severe natural threat—landslide (Kimmins, 2004). For certain animal species it leads to habitat fragmentation and loss (Fahrig, 1999), because some specific habitats cannot be maintained alongside timber production. Biodiversity-oriented management can help to preserve not only red-listed boreal forest species, but also to maintain strong and healthy ecosystems (Tikkanen et al., 2006).

---

* Corresponding author.
E-mail addresses: oksana.skaldina@uef.fi (O. Skaldina), jouni.sorvari@uef.fi (J. Sorvari).

http://dx.doi.org/10.1016/j.ecolind.2016.08.039

Biomonitoring is an essential part of sustainable environment management and the search for biological/ecological indicators and biomarkers, which promote better understanding for ecosystem well being is highly demanded. Ecological indicators as a subset of environmental indicators provide insight into the state and dynamics of biological systems (National Research Council, 2000). Biomarkers are crucial tools for revealing early warning signals of environmental change before the onset of irreversible damage at the population level (Amiard-Triquet et al., 2013). Ants represent proper indicator group to monitor environmental changes from individual-based to population-based levels, besides they possess important functional role in ecosystems, they also have small size, high individual sensitiveness to climate and microclimate change and long life-span of the colonies (Folgarait, 1998; Kaspari and Majer 2000; Christ, 2009). They are opportunistic objects to be reliable environmental, ecological and biodiversity indicators in cold biomes of the northern hemisphere (Ellison, 2012). In European boreal and temperate coniferous forests, red wood ants of the *Formica rufa* group are territorial and ecological dominants of the terrestrial environments. The ecological effect of these insects is extended over several trophic levels and they can be considered environmental engineer species due to their ability to change soil properties, e.g. nutrient and pH levels and maintain constant temperature inside the nest, about 10°C higher then ambient temperature (Frouz and Jilkova, 2008).

The most abundant and common ant in boreal forests in Finland is the highly polygynous (multiple queens) and polydomous (multiple nests) species *Formica aquilonia* (Yarrow, 1955) (Punttila and Kilpeläinen, 2009). The species is classed as Near Threatened by the International Union for Conservation of Nature (IUCN, 2015). Clearcutting can cause severe effects on forest-dwelling populations of *F. aquilonia.* It leads to a) reduction of nest mound size, b) decrease in sexual offspring production and bias in sex ratio, c) production of smaller workers with lower body energy reserves, d) increased rate of nest abandonment, and e) increase in the aggressiveness between neighbouring colonies causing decline in polydomous relationships among colonies (Sorvari and Hakkarainen, 2004, 2005, 2007a,b,c, 2009). Consequently, these changes diminish the ecological significance of this species in the forest ecosystem and weaken the ecosystem itself. The current degree of studies on survival strategies, adaptive capacity of *F. aquilonia* and necessary conservation actions for this species in terms of the present economic development of taiga remains insufficient. Thus, further studies of its adaptability and variability under different levels of disturbance and the search for reliable biomarkers of its well being in natural and disturbed habitats is highly in demand.

Like other red wood ants, *F. aquilonia* possesses diversity of red-brownish melanin-based conspicuous cuticular colouration. Studies of the colouration traits of this ant species can contribute to new ecological indicators and biomarkers of environmental change. Both genetic and environmental cues are involved in an organism development to fit its phenotype to selective conditions (Leimar, 2009). Consequently, phenotypic variability and polymorphism are good sources for morphological traits that are sensitive to environmental stress and useful for both short- and long-term biomonitoring programs. Concerning inter-individual variation in the degree of melanin-based coloration in ants it is worth considering that melanin is endogenously produced, hence this variability often possesses a non-negligible component. Condition-dependent or environmentally induced component of colour expression is more pronounced in invertebrates than vertebrates (Roulin, 2016). For this reason, melanin coloration could be used as a phenotypic plasticity marker of stress as invertebrate individuals living in stressful environments may develop a darker coloration comparably to those from benign habitats. Variability of size, pilosity, petiole shape, mandible dentition and colouration of *F. aquilonia* workers were shown to be good predictors for: analysis of spatial population structure, distinguishing of the period in the life of settlement (growth and dispersal, stabilization, depopulation and defragmentation), studies of species reaction on different kinds of disturbance (Gilev, 2003; Fedoseeva, 2011; Korochkina et al., 2014; Gilev et al., 2015).

Most studies on phenotypic variations of *F. aquilonia* were conducted on workers, and there are few studies about queens that are the main factor for genetic diversity of the ant's family. Unfertilized young queens (gynes) could be useful for such studies for several reasons: a) same conditions of rearing within the same nest; b) same age; c) originating from the particular nest and not migrating from the other nests. It is shown that the sensitivity of body size variations of *F. aquilonia* queens to environmental change may vary over time since the disturbance; the body size seems to respond soon after disturbance by increase in head width (Haatanen and Sorvari, unpublished) but later the body size seems not to respond to the disturbance anymore (Haatanen and Sorvari, 2013).

Concerning insects, environmental stress may affect naturally selected traits, such as body size or shape (e.g., Sorvari and Hakkarainen, 2009), but usually the effects are remote and can be revealed in long-term monitoring programs (Fedoseeva, 2011). On the other hand, changes in colouration and melanisation provide rapid information about various environmental stresses, such as nutritional imbalance (Talloen et al., 2004; Lee et al., 2008) and thermal stress (Kemp et al., 2006), UV radiation (Debecker et al., 2015), pathogens (Wilson et al., 2001).

Insects' colouration as an environmentally sensitive trait, can be studied from three points of view: a) colouration variability and polymorphism as a consequence of phenotypic plasticity (Whitman and Agraval, 2009; Tibbetts et al., 2011); b) melanisation as a reflection of the physiological state, processes and adaptations (Trullas et al., 2007; Karl et al., 2010); c) symmetrical and asymmetrical traits as different adaptations and signals, and also fluctuating asymmetry as a measure of developmental stability (Jennions, 1998; Barabas and Hancock, 1999).

Both changes in melanisation and increase in fluctuating asymmetry can show the species reaction to changed conditions. Cuticular melanisation is a well known kind of phenotypic respond to such factors as amount and quality of food resources, fluctuations in population density, temperature and UV radiation, level of predation and parasites (Wilson et al., 2001; True, 2003; Talloen et al., 2004; Kemp et al., 2006; Rajpurohit et al., 2008; de Souza et al., 2011; Debecker et al., 2015). Insect cuticle is rather more a flexible biological system than inert substance, and it is an important interface between physiological processes inside an individual's body and environmental conditions (Neville, 1975; Andersen, 1979). Melanin pigments are significant components of an insect's cuticle (Fuzeau-Braesch, 1972). On the one hand, they are involved in conspicuous cuticular colouration and traits formation, and on the other hand, they play crucial roles in many physiological processes, such as thermoregulation and desiccation resistance. In addition, they are highly responsible for immune defence: encapsulation rate and melanisation of haemolymph (Vilmos and Kurucz, 1998; Tsakas and Marmaras, 2010). Interestingly, in vertebrates it was found that the degree of melanisation has been associated with fluctuating asymmetry as a female melanin-ornament signals offspring fluctuating asymmetry in the barn owl (Roulin et al., 2003).

Fluctuating asymmetry (FA) is an important indicator of the level of stress in the environment as signs of developmental instability become elevated well before severe effects on fitness components appear (Møller, 1998). Moreover, anthropogenic stressors generate lager effects on FA in comparison to naturally occurring stressors (Beasley et al., 2013). In addition, FA is considered a good predictor for finding reliable signs of developmental instability in ants (Kaspari and Majer, 2000).

The aim of the study is to reveal environmentally sensitive traits in cuticular colouration of *F. aquilonia* red wood ant gynes and search for the suitable ecological indicators of environmental stress in managed coniferous forests.

2. Materials and methods

2.1. Study area and study sample

The total of 128 pre-mating queens (gynes) of *F. aquilonia* ants (mean ± SD: 5.6 ± 3.2 individuals per nest) were collected before nuptial flight manually from inside or surface of 23 nests around the city of Jyväskylä (WGS84 coordinates 62.24°N, 25.62°E) in May 2001 and stored in 96% alcohol. In total 11 nests were sampled from natural, bilberry (*Vaccinium myrtillus* L.) growth forest interiors, where Norway spruce (*Picea abies* L.) over 70 years old was a dominant tree species. In addition, 12 nests were sampled from disturbed habitats, clear-cuts (5 nests) and their edges (7 nests) that had been logged 1–2 years before the sampling. Nests in forest edges were less than 15 m away from the clear-cut, i.e., inside the forest. The material was collected from eight different forest stands and six clear-cut stands (1–2 nests each stand).

2.2. Photographing

The head capsules of the gynes were dried and then fixed on the object-plate with the underside of the head (gula) down. All the photos of ant heads were taken using a digital stereo microscope Olympus SZ40 and DeltaPix Invenio 3S 3 Mpixel CMOS camera with magnification 35×, exposure 45 and illuminance 2800 lx. In total, 128 photos of *F. aquilonia* queen heads were taken. For processing the images, we used Adobe Photoshop CS5 and ImageJ (Schneider et al., 2012) softwares. For assessment of melanisation degree (MD) we converted photos into grey scale (Fig. 1A). For easier evaluation of colour variations and fluctuating asymmetry (FA) we converted the greyscale photos into negatives (Fig. 1B). This made pigmentation area more clearly visible and distinct.

2.3. Colour variation, melanisation degree and fluctuating asymmetry

We used the maximum head width above the eyes (Sorvari and Hakkarainen, 2009) as an assessment parameter of body size of *F. aquilonia* gynes. The width of the head was analysed using image analysis software (ImageJ). We provided categorization of face colour variations (morphs) using a descriptive methods of population-morphology approach (Gilev, 2003; Yablokov, 1976, 1986). Workers of *F. aquilonia* exhibit a black spot of variable shape and size on thorax (Gilev, 2002, 2015). However, in studied gynes, there was no variability in the shape of the black spot (mesonotum + scutellum), only slight variation in darkness of the red pigment in pronotum and propodeum was present.

In total analysis of melanisation degree (MD) has been made for 117 faces (11 photos were excluded due to insufficient quality for this kind of analysis—existence of cracks on the heads surfaces). On the face of a gyne two certain areas (rectangles) were picked up: upper part melanisation (UPM) and lower part melanisation (LPM) (Fig. 1A). These parts reflected specific features of colour morph variability of *F. aquilonia* gynes, and matched the border between darker and lighter parts. On each of these areas melanisation degree was measured (six times per individual) as the mean grey scale darkness of the pixels, i.e., 0–255 in ImageJ. Prior to the statistical analyses the scale was calibrated so that zero represented 0–completely white and 255–completely dark (Sorvari et al., 2008; Fedorka et al., 2013). The intra class correlation coefficient as a repeatability of the measurements was very high in both UPM and LPM (0.99 and 1.00, respectively).

For the evaluation of FA of colour patterns on the right and left sides of the face we analysed 70 faces, and 58 were excluded due to assessment inability or high melanisation rank. Among those 70 gynes 33 were from forest interiors, 12-from forest edges and 25-from clear-cut zones. According to classification of symmetrical objects (Graham et al., 2010) colour variations on faces of gynes were described as inconsistent symmetry (with no consistent topology, no homologous landmarks, and no matching points). Therefore, we used a modified method of continuous symmetry measure (CSM) (Zabrodsky et al., 1995; Milner et al., 2005). For each part (left/right) of the head we measured the length of 3 lines, conducted at a certain angle: 30°, 45° and 60° from middle of the lowest ocelli until the end of the black-pigmented area (Fig. 1B). For the level of FA we calculated the length difference (left-right) for each pair of lines for each individual. The length of the lines was measured in pixels and then converted into mm. To distinguish true asymmetry from measurement error (Swaddle et al., 1994; Tomkins and Kotiaho, 2001; Swaddle, 2003) the measurements were provided 6 times for each axis, and then the mean values were taken. The angles were set manually on a pixel field, thus the angle of each line contained some deviation (sd) around the angle targeted. However, the deviations of each angle (30°, 45° and 60°) between the left and right side did not differ between disturbed habitats and forest interiors (*P*-values = 0.69–0.99).

2.4. Statistics

The repeatability analyses for melanisation parameters were carried out using reliability tests in SPSS 21 statistical software. The statistical analyses were carried out using SAS 9.4 statistical software (SAS Institute Inc.). Frequency of different colour morphs was compared using likelihood ratio chi square test. The differences in darkness and fluctuating asymmetry were analysed using linear mixed models. Head width of individuals was used as a covariate in the models. In addition, since several individuals from the same nests were studied, the nest of origin was included as a random factor in the models.

3. Results

3.1. Colour morphs

The analysis revealed colour variability on faces, propodeum and abdomen of *F. aquilonia* gynes, while distinct shapes or clearly visible colour morphs were found only on faces. We consider this type of colour variability to be more of continuous origin, differing from true polymorphism, which it is always discrete, discontinuous and strongly bimodal or polymodal (Gray and McKinnon, 2007). Despite, several traits of continuous variability can be distinguished as alternative, discrete, quantitative signs—phenes (Yablokov, 1976), there should be a significant proof of appearance of various alleles of a single gene to consider a trait to be a real phene (Yablokov, 1986). However recent studies concerning heritability of colour in *Formica rufa* workers revealed red wood colouration to be more environmentally dependent than genetically predicted (Skaldina and Sorvari, unpublished). Thereafter we use term “morph” rather than “phene”. Specificity of colour variations, changes in amount and direction of pigment spread in *F. aquilonia* gynes's faces are rather directed than stochastic, thus it is possible to set some threshold ranks and define classes of categorisation.

The categorisation revealed variable, clearly visible, symmetrical (left/right) colour morphs with thresholds of pigment spread

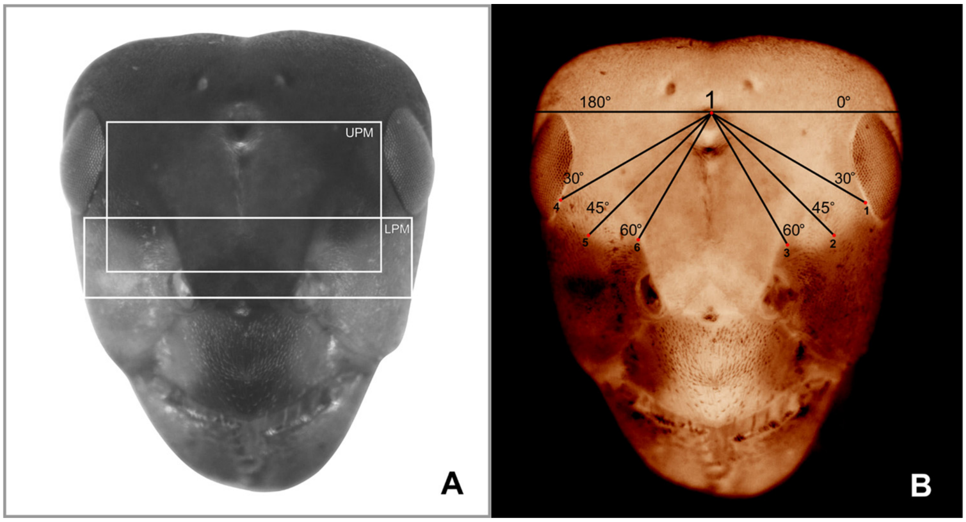


Fig 1. A. Assessment of melanisation degree (MD) on the rectangle areas: UPM (the matching points are the lowest frontal ocelli, inner margins of compound eyes and upper edge of antennas insertions) and LPM (the matching points are the lowest points of compound eyes, visible margins of the cheeks and lower edge of antennas insertions). B. Assessment of fluctuating asymmetry (FA): lines, conducted at 30°, 45°, 60°.

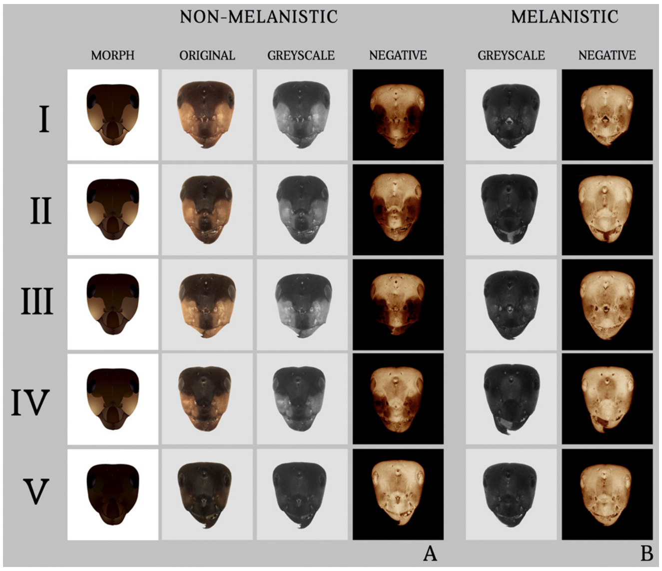


Fig. 2. Classes of non-melanistic (A) and melanistic (B) morphs on the *F. aquilonia* gynes faces in accordance with shapes of pigment spread and border between dark and light parts. I threshold rank – the border is visibly clear, the line is curved and touches eyes in the middle part; II threshold rank – the border is visibly clear, the line is more straight than curved, it touches eyes in the lower part; III threshold rank – the border is visibly clear, the line with small convexities, it touches eyes in the lower part; IV threshold rank – the border is visibly clear, convexities are big and spread on cheeks; V threshold rank – the border isn't visibly clear, line couldn't be identified.

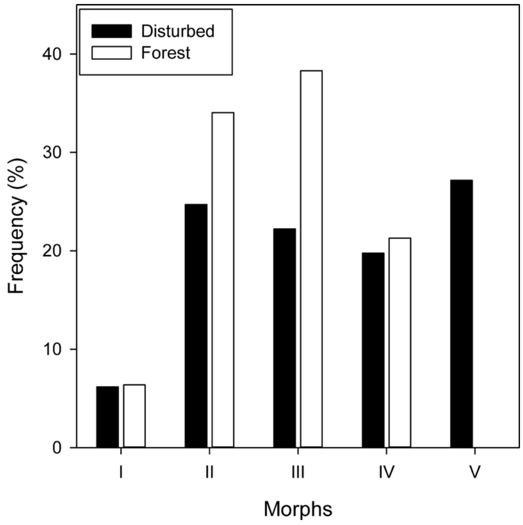


Fig. 3. Frequencies of five groups (I–V) of colour morphs of *F. aquilonia* gynes' faces in natural and disturbed habitats.

on the gynes faces (Fig. 2A). When we converted photos into negatives, it was possible to distinguish distinct colour variations even on highly melanised individuals, while on unprocessed photos they were not clearly visible (Fig. 2B). Gynes with totally dark cuticular colouration and absence of a clear visible border between pigmented areas were set to the group of morph V. The difference between dark morph V and melanistic individuals from all classes of morphs (I–IV) was visible only on the negative photos (Fig. 2B).

The frequency of the morph distribution was significantly different between forest habitats and disturbed habitats (likelihood ratio chi square test: df = 4, $G^2 = 23.71$, P < 0.0001; Fig. 3). This difference was caused by the absence of morph V in forest habitat nests. When the frequencies were compared without type V the morph frequencies did not differ any more (df = 3, $G^2 = 0.99$, P = 0.80). Interestingly, the colour morph frequencies were significantly different between the clear-cuts and forest edges (both included into disturbed habitats; df = 4, $G^2 = 15.53$, P = 0.004). Again, when the colour morph V was removed, the frequency distribution was no longer significant (df = 3, $G^2 = 7.34$, P = 0.06).

3.2. Melanisation

The habitat type and head width of the gynes were associated with the MD of LPM of the gyne's face (habitat type: $F_{1,21.1} = 11.90$, P = 0.0024; head width: $F_{1,95.5} = 9.36$, P = 0.0029). The gynes were darker in disturbed habitats (clear-cuts and clear-cut edges) than in forests (mean ± 95% CL, disturbed habitats: 174.3 ± 10.2, forests: 149.7 ± 10.7). The MD of the UPM of the face was also associated with the habitat type (habitat type: $F_{1,21.4} = 9.27$, P = 0.0061). Although gynes from disturbed habitats were darker (disturbed habitats: 190.4 ± 4.4, forests: 181.0 ± 4.7) the MD in the UPM of face was not significantly dependent on size (head width: $F_{1,97.4} = 0.76$, P = 0.39).

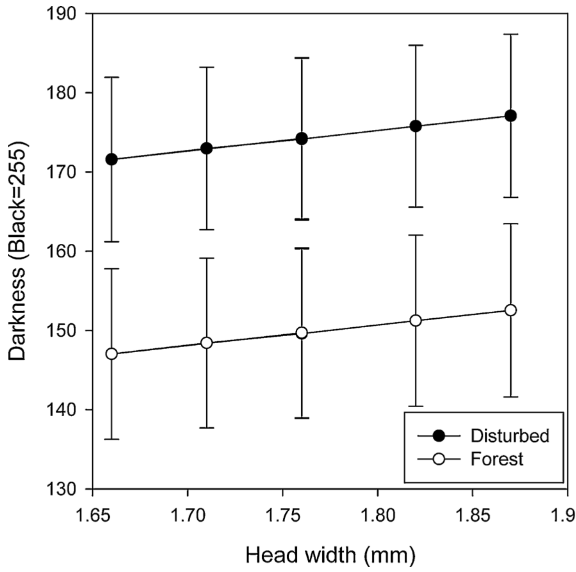


Fig. 4. Similarity in melanisation degree of *F. aquilonia* gynes per each colony in all habitat types (N = 117). The darkness values are scaled grey value from ImageJ software.

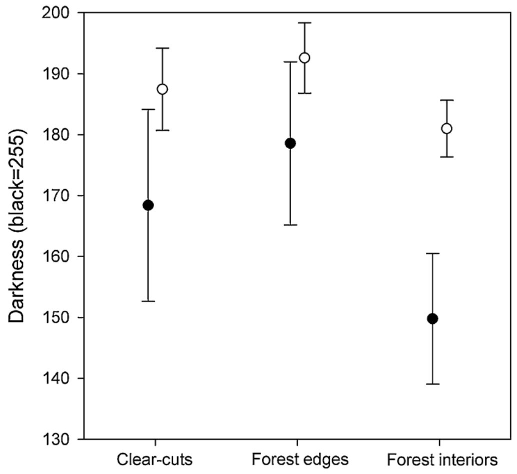


Fig. 5. Mean darkness of *F. aquilonia* gynes' faces in clear-cuts, edges and forest interiors. Filled symbols: LPM, open symbols: UPM. The darkness values are scaled grey value from ImageJ software.

Gynes from the same nest tend to have a similar MD of LPM (Fig. 4). When clear-cuts and edges were treated as a separate habitat type the habitat type affected the MD of LPM and UPM but so that the edges and forests differed significantly whereas the clear-cuts did not differ from the other habitat types; however, the mean darkness clear-cut individuals was intermediate to those from edges and forests (Tukey's test, edge vs. clear-cut: $P_{LPM} = 0.57$, $P_{UPM} = 0.24$, edge versus forest: $P_{LPM} = 0.0061$, $P_{UPM} = 0.004$, clear-cut vs. forest: $P_{LPM} = 0.13$, $P_{UPM} = 0.12$; Fig. 5).

The MD of the LPM of the face increased with an increase in head width (Fig. 6). The association between head width and the LPM was similar between the habitat types (interaction term habitat × head width: $F_{1,94.8} = 0.14$, $P = 0.71$). In both natural and disturbed habitats, the association between head width and MD of the UPM of the face was also similar (interaction term habitat × head width: $F_{1,96.7} = 3.22$, $P = 0.076$).

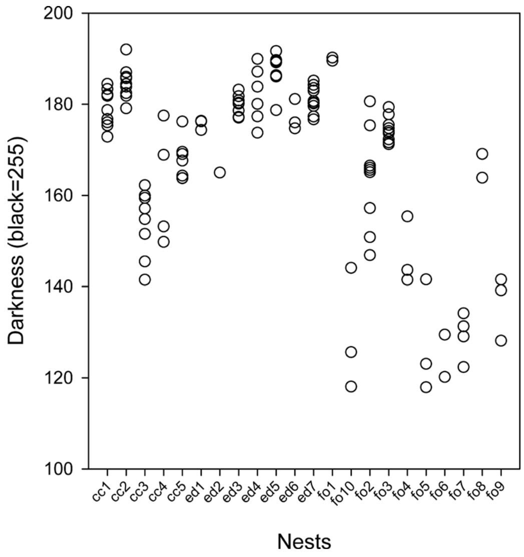


Fig. 6. Association between MD of LPM and head width of *F. aquilonia* gynes in natural and disturbed habitats. The darkness values are scaled grey value from ImageJ software.

3.3. Fluctuating asymmetry

The analysis revealed some degree of fluctuating asymmetry (FA) of continuous symmetrical colour variations between the right and left sides of *F. aquilonia* gynes' faces (mean ± SD: 30°: 0.047 ± 0.028; 45°: 0.046 ± 0.055; 60°: 0.033 ± 0.043). However, the amount of FA in face colour variations did not differ between disturbed habitats and forests (30°: $F_{1,66} = 0.01$, $P = 0.94$; 45°: $F_{1,66} = 0.71$, $P = 0.71$; 60°: $F_{1,66} = 1.36$, $P = 0.25$). In addition, the head width did not affect the amount of FA (30°: $F_{1,66} = 0.52$, $P = 0.47$; 45°: $F_{1,66} = 0.85$, $P = 0.36$; 60°: $F_{1,66} = 1.45$, $P = 0.23$). The effect of head width on the amount of FA was similarly non-significant between disturbed habitats and forests (interaction term habitat × head width, 30°: $F_{1,65} = 0.09$, $P = 0.76$; 45°: $F_{1,65} = 0.23$, $P = 0.63$; 60°: $F_{1,65} = 0.32$, $P = 0.57$).

In addition, linear mixed model analysis revealed no significant associations between melanisation measurements and FA measurements; UPM, 30°: $F_{1,50.6} = 0.32$, $P = 0.57$; 45°: $F_{1,49.6} = 2.68$, $P = 0.11$; 60°: $F_{1,49.4} = 1.81$, $P = 0.19$; LPM, 30°: $F_{1,49.5} = 3.45$, $P = 0.07$; 45°: $F_{1,48.9} = 3.21$, $P = 0.08$; 60°: $F_{1,48.8} = 1.71$, $P = 0.20$.

4. Discussion

In *F. aquilonia* gynes from Finnish population colour morphs are revealed on the faces, while on the other body parts variability is just continuous or not present at all. These results may exhibit the reflection of an adaptation capacity of this species, since melanin-based colour polymorphism is considered a marker of genetic adaptations (Gray and McKinnon, 2007), and polymorphic species are known to be more resilient to environmental stress comparably to monomorphic ones (Whitman and Agraval, 2009; Roulin, 2014). Melanin production has a significant genetic component as genes involved into melanogenesis can pleiotropically regulate other traits such as resistance to stress (Ducrest et al., 2008). Consequently, discovered phenotypic polymorphism on the gynes faces enables search of ecological indicators on these body parts.

Natural and disturbed habitats differed significantly in morphs distribution, but when highly melanised individuals of the V morph group were excluded, no differences were found. Furthermore, clear-cuts and forest edges, as disturbed habitats, differed by the frequency of the darkest morph V. Such distribution of morphs may represent natural variability of the species. At the same time, highly melanised phenotypes might show the species response to changed environmental conditions. As genetic relatedness among gynes is generally very low in this species, thus it is highly probable, that they are not sisters (Sundström et al., 2005). Therefore, the tendency for similar degree of melanisation in individuals from the same nest gives additional support for the prediction of environmental melanisation. Taking into account pleiotropic basis of melanin-based colouration it is also possible that ants possessing genes encoding for a dark colour may be selected in stressful habitats because the same genes also encode for other phenotypes useful to resist stress.

In studied *F. aquilonia* gynes both upper and lower parts of the melanin-based colour morphs were associated with the habitat type, and in clear-cuts and forest edges the enhanced melanisation degree revealed. It is known, that melanin formation in insect cuticle can be innate, associated with the molt, or spontaneously induced in imago by various environmental factors (Sugumaran, 2002; Lee et al., 2008; de Souza et al., 2011; Roulin, 2014; Debecker et al., 2015). In addition, larval stressors are able to bridge metamorphosis and have the carryover effects in adults (Pechenik, 2006; Debecker et al., 2015). As the studied gynes were collected one year after the clear-cutting, they might have faced environmental stress during larval and pupal stages. As an environmental stress, clear-cutting causes numeric changings to the forest-dwelling ants. For instance, it exposes ant nests to direct sunlight and wind, produces more unstable and generally drier nest inside microclimate and lower temperatures relative to ambient (Sorvari and Hakkarainen, 2009; Sorvari et al., 2016). Large nests of *F. aquilonia* are able to maintain stable temperature, whereas in small ones the temperature may vary more due to the lack of capacity for internal temperature regulation (Rosengren et al., 1987). Thus, for the ants there might be a strong need for physiological adaptations at the organismal level.

Highly melanised insects are capable for better thermoregulation (True, 2003) and in some cases tolerate desiccation more easily (Rajpurohit et al., 2008). It is also possible, that increased cuticular melanisation interrelated with enhanced immune response. Melanisation-encapsulation rate is an innate immune response in insects, which in these organisms shows no memory, but is rapid, lasting up to a few days and offering powerful resistance to microbial infections (Gillespie et al., 1997; Vilmos and Kurucz, 1998). Gynes of *F. aquilonia* from clear-cut areas possess an enhanced encapsulation reaction indicating decreased susceptibility to diseases and parasites (Sorvari et al., 2008).

Our results revealed that the most heavily melanised gynes were from forest edges. Forest edges might provide some benefits for the ants, even when the anthills are located near disturbed habitats. In clear-cuts the probability of sexual offspring production and nest survival is higher among nests in a forest edge zone (distance to the forest edge <9.2 m) compared to the clear-cut centre zone (Sorvari, 2013). In addition, inside undisturbed ecosystems forest edges were the best habitat types, providing the rich food resources for the Canadian ant *Formica podzolica* (Deslippe and Savolainen, 1994, 1995). Ambient conditions may favour increase in melanisation. Melanins are costly to produce (Talloen et al., 2004; Roulin, 2016), and high-quality protein diet produces heavily melanised cuticles in insects (Lee et al., 2008). In an experimental study with the fire ant *Solenopsis invicta* it had been shown, that poor, insect free, diet induced nonmelanized cuticle (Williams et al., 1987). Finally, presence of more heavily melanised ants in disturbed habitats can be explained with two non-mutually exclusive scenarios. At the one hand, melanins are produced endogenously and thus their expression varies among individuals and strongly depends on the ambient. At the other hand, being regulated by pleiotropic mechanisms, melanins can signal about presence of some other traits useful for stress resilience. To summarize, degree of melanisation, measured on the faces (an indicator body parts) can testify the species adaptability to changed environmental conditions, contributing to better thermoregulation, desiccation resistance and better immune response. Either it can be induced by the enhanced nutrition, but further observations are highly needed.

Symmetry is closely interconnected with developmental stability – the ability to control development under given environmental conditions and fluctuating asymmetry shows deviations, caused by environmental stresses or genetic problems (or both of them in the same time) from a regular phenotype and thus reveals information on developmental instability (Møller, 1998). No significant differences in FA of face colouration in *F. aquilonia* in natural and disturbed habitats were revealed. In addition, we did not find associations between FA parameters and melanisation degrees. It is well known, that developmental instability could be induced by genetic factors (inbreeding, homozygosity, hybridization, mutation) and/or environmental factors (temperature, food, pollutants, population density, sound, light, parasites) (Møller, 1998). In our study under the given level of disturbance in Finnish managed coniferous forests (single cause—clear-cutting) no signs of developmental instability of colouration traits were revealed. At the same time, revealing FA of left/right colouration variations may be useful for further studies of adaptation capacity of red wood ants under more severe or combined natural and anthropogenic disturbances, such as landslides, wild fires, heavy metal and organic pollutants contamination.

It is well known, that size could mask or alter potential relationships between colouration, asymmetry and the dependent variables, thus taking account of size is always necessary (Swaddle, 2003). In the studied samples, no interconnection between body size and asymmetry of colour variations exists. At the same time both in natural and disturbed habitats, gynes have a tendency to higher melanisation with the size increment. Among ant queens, large body size has been associated with better survival capacity, better immune defence, and higher reproductive success (Wiernasz and Cole, 2003; Sorvari et al., 2008; Enzmann et al., 2014). In some conditions, melanisation is also well known to be beneficial, when selection on cuticle darkness indirectly favours the pathogen resistance (Fedorka et al., 2013). Revealing a more precise interconnection between size and melanisation of red wood ants in managed forests requires further studies.

In contemporary environmental management a variety of bioindicators, biomonitors and biomarkers are used (Markert et al., 2003). Biomarkers of defence show a variety of defence mechanisms, allowing the survival of organisms in a degraded environment and biomarkers of damage reveal more or less severe biological impairments (Amiard-Triquet et al., 2013). Ecological indicators include whole set of organisms being used to access the condition of the environment (Dale and Beyeler, 2001). Although in addition to measurement attributes, on which biomarkers are usually concentrated, they also require ecological validation (McCarty et al., 2002).

Habitat loss has greater effect on population persistence than habitat fragmentation (change in habitat configuration) and population survival may show a threshold response to habitat loss (Fahrig, 1999). Revealing early signs of the degradation processes in wild species populations under different levels of disturbance is an important task for nature protection and conservation. Informative and conspicuous ecological indicators, such as melanisation degree and possibly fluctuating asymmetry of colour morphs, can contribute into revealing early signs of this threshold response to different levels of disturbance and allow swift action for species protection and conservation.

5. Conclusions

Our results indicate that colouration of forest-dwelling red wood ants gynes could be a good source for the search for ecological indicators, and further on—biomarkers of population well-being in natural and transformed habitats under different levels of disturbance. Highly melanised individuals, especially the morph V, might be considered as indicators of environmental change. Some additional studies are necessary to test, whether melanisation degree of the face of *F. aquilonia* gynes and workers could be used as a biomarker of defence. Measurable fluctuating asymmetry in face colour patterns as a colouration trait has a potential to serve as a biomarker of damage in severely disturbed habitats. Red wood ants of the *Formica rufa* group inhabit northern coniferous and temperate forests in Eurasia and North America, thus provide a possible biomarker tool in a wide geographic area. However, while our results are promising, the usefulness of different species within the species group and studies provided on workers needs further efforts.

Acknowledgements

We are grateful to Marja-Katariina Haatanen for kindly taking photos of the *F. aquilonia* gynes and Nataliia Buzhenina for assistance in artwork for figures. We are also grateful to the late Harri Hakkarainen for his help and company during the collection of field data. We thank Alexandre Roulin and an anonymous reviewer for their useful comments, which helped to improve the manuscript. Daniel Blande provided comments on the structure and language of the manuscript. The study was financially supported by the grants from the Centre for International Mobility (CIMO) and Kuopio Naturalists Society (KLYY).

References

Andersen, S.O., 1979. Biochemistry of insect cuticle. Ann. Rev. Entomol. 24, 29–61.

Amiard-Triquet, C., Amiard, J.-C., Rainbow, P.S. (Eds.), 2013. CRC Press, Boca Raton, FL, USA.

Barabas, S.P., Hancock, E.G., 1999. Asymmetrical colour and wing-folding in *Tithrone roseipennis* (Saussure 1870) a neotropical praying mantis (Mantodea Hymenopodidae). Trop. Zool. 12 (2), 325–334.

Beasley, D.E., Bonisoli-Alquati, A., Mousseau, T.A., 2013. The use of fluctuating asymmetry as a measure of environmentally induced developmental instability: a meta-analysis. Ecol. Indic. 30, 218–226.

Christ, T.O., 2009. Biodiversity, species interactions, and functional roles of ants (Hymenoptera: Formicidae) in fragmented landscapes: a review. Myrmecol. News 12, 3–13.

Dale, V.H., Beyeler, S.C., 2001. Challenges in the development and use of ecological indicators. Ecol. Indic. 1, 3–10.

Debecker, S., Sommaruga, R., Maes, T., Stoks, R., 2015. Larval UV exposure impairs adult immune function through a trade-off with larval investment in cuticular melanin. Funct. Ecol. 29, 1292–1299.

de Souza, D.J., Devers, S., Lenoir, A., 2011. *Blochmannia* endosymbionts and their host, the ant Camponotus fellah: cuticular hydrocarbons and melanization. C. R. Biol. 334, 737–741.

Deslippe, R.J., Savolainen, R., 1994. Role of food supply in structuring a population of *Formica* ants. J. Anim. Ecol. 63, 756–764.

Deslippe, R.J., Savolainen, R., 1995. Sex investment in a social insect: the proximate role of food. Ecology 76 (2), 375–382.

Ducrest, A.-L., Keller, L., Roulin, A., 2008. Pleiotropy in the melanocortin system, coloration and behavioural syndromes. Trends Ecol. Evol. 23, 502–510.

Ellison, A.M., 2012. Out of Oz: opportunities and challenges for using ants (Hymenoptera: Formicidae) as biological indicators in north-temperate cold biomes. Myrmecol. News 17, 105–119.

Enzmann, B.L., Gibbs, A.G., Nonacs, P., 2014. The cost of being queen: investment across *Pogonomyrmex* harvester ant gynes that differ in degree of claustrality. J. Insect Physiol. 70, 134–142.

Fahrig, L., 1999. Forest lost and fragmentation: which has the greater effect on persistence of forest-dwelling animals? In: Rochelle, J.A., Lehmann, L.A., Wisniewski, J. (Eds.), Forest Fragmentation: Wildlife and Management Implications. Brill, Leiden, pp. 87–95.

Fedorka, K.M., Lee, V., Winterhalter, W.E., 2013. Thermal environment shapes cuticle melanism and melanin-based immunity in the ground cricket *Allonemobius socius*. Evol. Ecol. 27, 521–531.

Fedoseeva, E.B., 2011. Morphometric characteristics of *Formica aquilonia* ants in monitoring of their settlements. Entomol. Rev. 91 (2), 152–168.

Folgarait, P.J., 1998. Ant biodiversity and its relationship to ecosystem functioning: a review. Biodivers. Conserv. 7, 1221–1244.

Frouz, J., Jilkova, V., 2008. The effect of ants on soil properties and processes (Hymenoptera: Formicidae). Myrmecol. News 11, 191–199.

Fuzeau-Braesch, S., 1972. Pigments and color changes. Annu. Rev. Entomol. 17, 403–424.

Gilev, A.V., Mershchiev, A.V., Malyshev, D.S., 2015. Interpretation of postglacial recolonization of *Formica aquilonia* (Hymenoptera, Formicidae) in Fennoscandia according to workers' color variation. Entomol. Rev. 95 (8), 941–946.

Gilev, A.V., 2003. The population pattern of the ant *Formica aquilonia* (Hymenoptera, Formicidae) in the Middle Urals. Usp. Sovrem. Biol. 123 (3), 223–228.

Gillespie, J.P., Kanost, M.R., Trenczek, T., 1997. Biological mediators of insect immunity. Annu. Rev. Entomol. 42, 611–643.

Graham, J.H., Raz, S., Hel-Or, H., Nevo, E., 2010. Fluctuating asymmetry: methods, theory, and applications. Symmetry 2 (2), 466–540.

Gray, S.M., McKinnon, J.S., 2007. Linking color polymorphism maintenance and speciation. Trends Ecol. Evol. 22 (2), 71–79.

Haatanen, M.K., Sorvari, J., 2013. Similarity of body size in queens of the wood ant *Formica aquilonia* from optimal and sub-optimal habitats indicates a strong heritable component. J. Insect Sci. 13 (115), 1–11.

IUCN, 2015. The IUCN Red List of Threatened Species. Version 2015.2, www.iucnredlist.org. Downloaded on 16 August 2015.

Jennions, M.D., 1998. Tibial coloration, fluctuating asymmetry and female choice behaviour in the damselfly *Platycypha caligata*. Anim. Behav. 55, 1517–1528.

Karl, I., Hoffmann, K.H., Fischer, K., 2010. Cuticular melanisation and immune response in a butterfly: local adaptation and lack of correlation. Ecol. Entomol. 35, 523–528.

Kaspari, M., Majer, J.D., 2000. Using ants to monitor environmental change. In: Agosti, D., Majer, J.D., Alonso, L.E., Schultz, T.R. (Eds.), Ants: Standard Methods for Measuring and Monitoring Biodiversity. Smithsonian Institution Press, Washington D.C, pp. 89–98.

Kemp, D.J., Vukusic, P., Rutowski, R.L., 2006. Stress-mediated covariance between nano-structural architecture and ultraviolet butterfly coloration. Funct. Ecol. 20, 282–289.

Kimmins, J.P., 2004. Forest Ecology: A Foundation for Sustainable Forest Management and Environmental Ethics in Forestry. Pearson Prentice Hall, Upper Saddle River, New Jersey.

Korochkina, N.I., Konopleva, E.E., Zryanina, T.A., 2014. Population structure of *Formica aquilonia* (Hymenoptera, Formicidae) at the boundary of boreal and subboreal landscapes in the Volga river region. Entomol. Rev. 94 (5), 664–674.

Laurance, W.F., 2010. Habitat destruction: death by a thousand cuts. In: Sodhi, D., Ehrlich, P. (Eds.), Conservation Biology for All. Oxford University Press, UK, pp. 73–87.

Lee, K.P., Simpson, S.J., Wilson, K., 2008. Dietary protein-quality influences melanisation and immune function in an insect. Funct. Ecol. 22, 1052–1061.

Leimar, O., 2009. Environmental and genetic cues in the evolution of phenotypic polymorphism. Evol. Ecol. 23, 125–135.

Møller, A.P., 1998. Developmental instability as a general measure of stress. Adv. Study Behav. 27, 181–213.

Markert, B.A., Breure, A.M., Zechmeister, H.G. (Eds.), 2003. Elsevier, Amsterdam.

McCarty, L.S., Power, M., Munkittrick, K.R., 2002. Bioindicators versus biomarkers in Ecological risk assessment. Hum. Ecol. Risk Assess. 8 (1), 159–164.

Milner, D., Her-Or, H., Keren, D., 2005. Analyzing symmetry in biological systems. Proc. IEEE Int. Conf. Image Proc. 1, 361–364.

National Research Council, 2000. Ecological Indicators for the Nation. National Academy Press, Washington, DC.

Neville, A.C., 1975. Biology of arthropod cuticle. Zoophysiol. Ecol. 4 (5), 445–453.

Pechenik, J.A., 2006. Larval experience and latent effects—metamorphosis is not a new beginning. Integr. Comp. Biol. 46, 323–333.

Pimm, S.L., Raven, P., 2000. Biodiversity: extinction by numbers. Nature 403, 843–845.

Punttila, P., Kilpeläinen, J., 2009. Distribution of mound-building ant species (Formica spp., Hymenoptera) in Finland: preliminary results of a national survey. Ann. Zool. Fenn. 46, 1–15.

Rajpurohit, S., Parkash, R., Ramniwas, S., 2008. Body melanisation and its adaptive role in thermoregulation and tolerance against desiccating conditions in drosophilids. Entomol. Res. 38, 49–60.

Rosengren, R., Fortelius, W., Lindström, K., Luther, A., 1987. Phenology and causation of nest heating and thermoregulation in red wood ants of the *Formica rufa* group studied in coniferous forest habitats in southern Finland. Ann. Zool. Fenn. 24, 147–155.

Roulin, A., Ducrest, A.-L., Balloux, F., Dijkstra, C., Riols, C., 2003. A female melanin-ornament signals offspring fluctuating asymmetry in the barn owl. Proc. R. Soc. B 270, 167–171.

Roulin, A., 2014. Melanin-based colour polymorphism responding to climate change. Global Change Biol. 20, 3344–3350.

Roulin, A., 2016. Condition-dependence, pleiotropy and the handicap principle of sexual selection in melanin-based colouration. Biol. Rev. 91, 328–348.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9 (7), 671–675.

Sorvari, J., 2013. Proximity to the forest edge affects the production of sexual offspring and colony survival in the red wood ant *Formica aquilonia* in forest clear-cuts. Scand. J. For. Res. 28 (5), 451–455.

Sorvari, J., Hakkarainen, H., 2004. Habitat-related aggressive behavior between neighboring colonies of the polydomous wood ant *Formica aquilonia*. Anim. Behav. 67, 151–153.

Sorvari, J., Hakkarainen, H., 2005. Deforestation reduces nest mound and decreases the production of sexual offspring in the wood ant *Formica aquilonia*. Ann. Zool. Fenn 42, 259–267.

Sorvari, J., Hakkarainen, H., 2007a. Forest clearing and sex ratio in forest-dwelling wood ant *Formica aquilonia*. Naturwissenschaften 94, 392–395.

Sorvari, J., Hakkarainen, H., 2007b. The role of food and colony size in sexual offspring production in a social insect: an experiment. Ecol. Entomol. 32, 11–14.

Sorvari, J., Hakkarainen, H., 2007c. Wood ants are wood ants: deforestation causes population declines in the polydomous wood ant *Formica aquilonia*. Ecol. Entomol. 32, 707–711.

Sorvari, J., Hakkarainen, H., 2009. Forest clear-cutting causes small workers in the polydomous wood ant *Formica aquilonia*. Ann. Zool. Fenn. 46, 431–438.

Sorvari, J., Hakkarainen, H., Rantala, M., 2008. Immune defense of ants is associated with changes in habitat characteristics. Environ. Entomol. 37 (1), 51–56.

Sorvari, J., Elo, R.A., Härkönen, S.K., 2016. Forest-built nest mounds of red wood ant *Formica aquilonia* are not good in clear fells. Appl. Soil Ecol. 101, 101–106.

Sugumaran, M., 2002. Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects. Pigment Cell Res. 15, 2–9.

Sundström, L., Seppä, P., Pamilo, P., 2005. Genetic population structure and dispersal patterns in *Formica* ants—a review. Ann. Zool. Fenn. 42, 163–177.

Swaddle, J.P., 2003. Fluctuating asymmetry, animal behavior, and evolution. Adv. Study Behav. 32, 169–205.

Swaddle, J.P., Witter, M.S., Cuthill, I.C., 1994. The analysis of fluctuating asymmetry. Anim. Behav. 48, 986–989.

Talloen, W., Van Dyck, H., Lens, L., 2004. The cost of melanization: butterfly wing coloration under environmental stress. Evolution 58 (2), 360–366.

Tibbetts, E.A., Skaldina, O., Zhao, V., Toth, A.L., Skaldin, M., Beani, L., Dale, J., 2011. Geographic variation in the status signals of *Polistes dominulus* paper wasp. PLoS One 6, e28173.

Tikkanen, O.P., Martikainen, P., Hyvärinen, E., Junninen, K., Kouki, J., 2006. Red-listed boreal forest species of Finland: associations with forest structure, tree species, and decaying wood. Ann. Zool. Fenn. 43, 373–383.

Tomkins, J.L., Kotiaho, J.S., 2001. Fluctuating asymmetry. In: Encyclopedia of Life Sciences. MacMillan Publishers Ltd., Nature Publishing Group, pp. 1–5.

True, J.R., 2003. Insect melanism: the molecules matter. Trends Ecol. Evol. 18 (12), 640–647.

Trullas, S.C., van Wyk, J.H., Spotila, J.R., 2007. Thermal melanism in ectoterms. J. Ther. Biol. 32, 235–245.

Tsakas, S., Marmaras, V.J., 2010. Insect immunity and its signaling: an overview. Invert. Surviv. J. 7, 228–238.

Vilmos, P., Kurucz, E., 1998. Insect immunity: evolutionary roots of the mammalian innate immune system. Immunol. Lett. 62, 59–66.

Wiernasz, D.C., Cole, B.J., 2003. Queen size mediates queen survival and colony fitness in harvester ants. Evolution 57 (9), 2179–2183.

Williams, D.F., Vander Meer, R.K., Logfren, C.S., 1987. Diet-induced nonmelanized cuticle in workers of the imported fire ant *Solenopsis invicta* Buren. Arch. Insect Biochem. Physiol. 4, 251–259.

Wilson, K., Cotter, S.C., Reeson, A.F., Pell, J.K., 2001. Melanism and disease resistance in insects. Ecol. Lett. 4, 637–649.

Whitman, D.W., Agraval, A.A., 2009. What is phenotypic plasticity and why is it important? In: Whitman, D.W., Ananthakrishnan, T.N. (Eds.), Phenotypic Plasticity in Insects: Mechanisms and Consequences. Science Publishers, Enfield, pp. 1–63.

Yablokov, A.V., 1976. Population morphology as a new trend in evolutionary-morphological and population research. J. Gen. Biol. 37 (5), 649–659.

Yablokov, A.V., 1986. Phenetics: Evolution, Population, Trait. Columbia University Press, New York.

Zabrodsky, H., Peleg, S., Avnir, D., 1995. Symmetry as a continuous feature. IEEE Trans. Pattern Anal. Mach. Intell. 17 (12), 1154–1166.