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DR SHARON ELIZABETH ZYTYSKA (Orcid ID : 0000-0002-0174-3303)

DR JÖRG MÜLLER (Orcid ID : 0000-0002-1409-1586)

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Minimal effects on genetic structuring of a fungus-dwelling saproxylic beetle after recolonization of a restored forest

Sharon E. Zytynska^{1*}, Inken Doerfler¹, Martin M. Gossner^{1,2}, Sarah Sturm¹, Wolfgang W. Weisser¹ and Jörg Müller^{3,4}

¹ *Technical University of Munich, Terrestrial Ecology Research Group, Department of Ecology and Ecosystem Management, School of Life Sciences Weihenstephan, Hans-Carl-von-Carlowitz-Platz 2, 85354 Freising, Germany*

² *Forest Entomology, Swiss Federal Research Institute WSL, Zürcherstrasse 111, 8903 Birmensdorf, Switzerland*

³ *Field Station Fabrikschleichach, University of Würzburg, Rauhenegrab, Germany*

⁴ *Nationalpark Bavarian Forest, Grafenau, Germany*

* corresponding author email: sharon.zytynska@tum.de

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Abstract

1. Habitat restoration aims to improve local habitat conditions for threatened species. While such restorations are widespread, rigorous evaluations of their success are rare. This is especially true of those considering species dynamics. Increasingly, deadwood is a target for forest restoration as many species directly and indirectly depend on this resource.
2. In a broadleaf forest in southern Germany, we explored the effect of landscape-wide deadwood restoration on the population genetic structure of the specialist fungus-dwelling saproxylic beetle *Bolitophagus reticulatus*. Before 2003, the northern area of this forest was intensively logged for more than half a century, while the southern part was less intensively managed. This drove populations of the host fungus *Fomes fomentarius*, and consequently the beetle, to local extinction in the northern part. Only after the first decade of restoration were both the fungus and its beetles present across all areas of the forest.
3. Using 17 newly-developed microsatellite loci, we show that these beetles exhibit population genetic structuring, mainly influenced by the north-south divide. However, the low degree of isolation-by-distance, and the low relatedness of beetles collected from the same trees or fungus, shows that strong dispersal ability is facilitating the recolonization of these forests on the scale of 10s km. In another ten years it is likely that the population will show even less genetic structuring.
4. *Synthesis and applications.* Through the recolonization of the fungus *Fomes fomentarius* and the fungus-dwelling beetle *Bolitophagus reticulatus* after deadwood restoration, we demonstrate that, while there are many discussions on the optimal spatial distribution of deadwood, just the presence of deadwood can be sufficient to enable recolonizations of specific species. As long as some relict populations of these

species are embedded in a once intensively managed forest, increased deadwood (amount and diversity) anywhere will benefit recolonization of the habitat. However, increasing deadwood diversity should also be encouraged to benefit even more species.

German Abstract

1. Renaturierung ist ein wichtiges Mittel zur Wiederherstellung von Lebensräumen bedrohter Arten. Überprüfungen solcher Maßnahmen sind allerdings eher selten. Totholz ist eines der Hauptziele bei der Renaturierung von Wäldern, da viele Organismen von diesem Substrat abhängen.
2. In dieser Studie wurde der Effekt einer landschaftsweiten Totholzanreicherung auf die Populationsgenetik eines auf Zunderschwamm-Pilze spezialisierten Käfers, dem Kerbhalsigen Zunderschwamm-Schwarzkäfer, untersucht. Im nördlichen Teil des Untersuchungsgebiets waren sowohl der Pilz als auch der Käfer auf Grund intensiver Holznutzung ausgestorben. Nach nur einem Jahrzehnt der bewussten Totholzanreicherung konnten sowohl der Pilz, als auch sein Bewohner, wieder flächendeckend nachgewiesen werden.
3. Mit Hilfe von 17 neuentwickelten Mikrosatelliten konnte gezeigt werden, dass die Käferpopulation hauptsächlich Nord-Süd strukturiert ist. Allerdings zeigt die geringe Struktur innerhalb der Population, dass die Art über ein enormes Ausbreitungspotential verfügt, welches eine Besiedlung auf der Skala von 10 km gut möglich macht. Die Ergebnisse lassen erwarten, dass in weiteren 10 Jahren die Struktur innerhalb der Population noch weiter abnehmen wird.
4. Die Wiederbesiedlung des einst ökologisch degradierten Nordens nach Totholzanreicherung durch den Zunderschwamm und auch seinen Bewohner, den Kerbhalsigen Zunderschwamm-Schwarzkäfer, zeigt, dass die oft diskutierte räumliche

Anordnung von Totholz weniger wichtig als die schlichte Präsenz dieser Struktur in einer Waldlandschaft ist. So lange Reliktpopulationen in einer Waldlandschaft vorhanden sind führt jede Anreicherung von Totholzmengen und –vielfalt zu einer Wiederbesiedlung ehemals ökologisch degradierter Waldflächen.

Keywords: *Bolitophagus reticulatus*; conservation planning; deadwood; *Fomes fomentarius*; fungi; microsatellites; population genetics; forest

Introduction

Habitat restoration is one of the key tools in the conservation of degraded landscapes. It aims to locally improve habitat conditions, particularly for threatened species. Increasing suitable habitat patches in a landscape allows species to increase their population size and reduces potential negative effects of habitat fragmentation. Such restorations are widely spread, however, rigorous evaluations on their success are rare (McKay *et al.* 2005; Maiorano, Falcucci & Boitani 2008).

The restoration of deadwood, and trees with specific microhabitats, in forests that have been used for production for centuries is one of the most common applications in forest conservation (Cavalli & Mason 2003; Lassauce *et al.* 2011; Larrieu *et al.* 2014). There is strong experimental evidence that saproxylic species [those that depend on dead or decaying wood, or are dependent on other organisms that are themselves dependent on dead wood] are positively influenced by increasing deadwood supply in all kind of forests (Seibold *et al.* 2015a). However, the effect of deadwood amount, spatial arrangement, and history, on the colonization success is still a matter of debate. One of the key questions here is the dispersal

ability of the various organisms living in dead and dying trees. For example, forest-based species with reduced dispersal ability (e.g. flightless beetles) were found to be less able to colonise isolated forest patches with an agricultural history (Buse 2012), and, in Finland, re-colonization of fire-restored forests by saproxylic species was greater in areas closer to more natural and fire-dominated landscapes (Kouki *et al.* 2012). However, in a forest experimental setup, Seibold *et al.* (2017) found little support for isolation effects in a forested landscape, beyond the limiting effect of the amount of available deadwood habitat for deadwood-dwelling beetles. The data in Seibold *et al.* (2017) is likely dominated by flight-active species, but it shows that we require greater understanding of how saproxylic beetles can disperse around the landscape. This will also benefit management strategy development, where it is important to know what drives the origin of new colonisations by saproxylic species in an area (see Drag & Cizek 2015). Here, one of the major challenges is with small or cryptic species since it is unclear if they have been previously overlooked, misidentified, or really have newly colonized an area. That species can persist in small numbers over a century without detection has been confirmed, e.g. for *Bius thoracicus* in the Pyrenees (Soldati, Barnouin & Noblecourt 2015) or *Danosoma fasciata* in the Bohemian Forest (Müller *et al.* 2010).

Under the current climate, beech-dominated forests form the majority of forested habitats in the temperate zone of Central Europe (Brunet, Fritz & Richnau 2010). This was not always the situation; beech forest habitats were drastically reduced in size circa 800 AD due to an increase in the human-mediated growth of more economically-valuable conifers (Gossner *et al.* 2013). This led to only small, isolated patches of beech forest remaining. More recently, beech management has led to a steady increase in beech forest areas, allowing for recolonization by dependent species that were previously locally extinct in small remnant

patches. However, removal of deadwood from these forests was still a common management practice, even up until the 2000's, and so there has been sustained extinction pressures on deadwood-dependent invertebrate species (e.g. Müller 2005b).

The specialist tenebrionid beetle *Bolitophagus reticulatus* lives on the deadwood-colonising fungus *Fomes fomentarius*, which is also host to numerous other arthropod species, in European beech forests (Thunes 1994; Thunes & Willassen 1997). *Bolitophagus reticulatus* beetles have previously been found to have good dispersal abilities using flight-mill experiments (Jonsson 2003) and experimentally exposed fungal sporocarps (Nilsson 1997). However, in the field, even at small scales of 25-30 m, the probability of the beetle to occur was reduced with increasing habitat isolation (Rukke & Midtgaard 1998; Sverdrup-Thygeson & Midtgaard 1998). In this case, despite the beetles exhibiting good dispersal capabilities under controlled experimental conditions, it is unclear if other factors may have led to dispersal limitation that contributed to reduced beetle occurrence on isolated trees, i.e. beyond just the lack of available habitat in the surrounding areas. Additionally, analysis of protein polymorphisms detected a strong genetic isolation pattern of habitat fragmentations on Norwegian populations of this beetle (Knutsen *et al.* 2000). With isolation effects limiting the dispersal of these beetles, a strategy involving the creation of 'stepping-stone' patches may need to be undertaken to enable recolonization of habitat fragments (Taylor *et al.* 1993). Using genetic tools, alongside historical information regarding habitat availability and information on dispersal abilities can provide useful information on which to base future conservation management plans (Segelbacher *et al.* 2010). In particular, they can provide important information about the power of restoration activities in fragmented landscapes.

In the beech-dominated forest landscape of the Steigerwald Forest, Germany, an unintended experiment of habitat removal was established in the mid-20th century (see Supporting Information: Appendix S1 for details). Here, up until 2003, all decaying or injured trees, and new colonisations of fungal sporocarps were removed, except in a few small patches where no management occurred (Müller 2005b). In the wake of this strategy, the fungus *F. fomentarius*, which is the principal decomposer of beech trees, became extinct in the northern part (Fabrikschleichach) of the Steigerwald Forest. As a consequence, the *Fomes* specialist beetle *B. reticulatus* was also locally extinct in this area (Müller 2005b). After 2003, a supply of deadwood was intentionally left in this part of the Steigerwald forest during logging operations (Müller 2005b). In 2004, young sporocarps of *Fomes* began to appear in a few areas (8% of forest beech plots; Müller 2005a; Müller 2005b); however, the *Fomes* specialist beetle *B. reticulatus* was not present since it requires larger decaying sporocarps. In contrast, in the southern parts of the forest numerous *Fomes* sporocarps (69% of beech forest plots) and *B. reticulatus* beetles could be found in both managed and unmanaged forest areas (Müller 2005a). During the following decade a massive increase of deadwood in the northern area happened as part of a forest conservation strategy (Doerfler *et al.* 2017). This is likely to have also encouraged the colonisation of *F. fomentarius* and consequently the beetle. Here, we sampled *B. reticulatus* across the northern and southern parts of the Steigerwald Forest to assess the occurrence of beetles in this area where, until a decade ago, the landscape had been without the fungus *F. fomentarius* for half a century (see Appendix S1 more details). We collected beetles from different trees, different fungi within the same tree, and also multiple individuals from the same fungus. We developed microsatellite markers after sequencing the genome of *B. reticulatus* to analyse their population genetic structure. First, we aimed to understand the patterns of isolation-by-distance and genetic relatedness between

beetle individuals across the three different scales (*within-fungi*, *within-tree*, and *among* trees). With strong dispersal limitation, we hypothesise that genetic relatedness among beetles would be higher at smaller spatial scales (e.g. *within-fungi*, *within-tree*) than at larger spatial scale (*among* trees). Second, we wanted to determine if the newly established population in the northern area was a random subset of genotypes from the regional gene pool (indicating a lack of dispersal limitation), or if colonisation occurred only from adjacent areas (dispersal limitation).

Materials and Methods

Study system and site

The study area is located in the ‘Steigerwald’ forest area in southern Germany (Bavaria) (N 49° 50' 53 E 10° 29' 41). Elevation varies between 300 to 450 m a.s.l. The mean annual temperature is 8.2 °C and mean annual rainfall is about 809 mm (DWD 2017). The study area is located in the forestry department Ebrach which is managed by the Bavarian State Forest Company and comprises 17,000 ha. The *managed* area covers 16,494.2 ha (97 % of the total forestry department). The remaining area is designated as six forest nature reserves, in which all management activities stopped 11-28 years ago (*unmanaged*). These reserves range from 24.9 ha to 183.4 ha in size. The majority of these areas were designated in the 1970ies and enlarged around 2010. The State Forest department is located in a landscape of agricultural land and private forests with large proportions of spruce (*Picea abies*) and pine (*Pinus sylvestris*).

The Bavarian State Forest Company has practiced an integrative conservation strategy in this area since 2006. This includes the abandonment of clear felling; a focus on tree species native to Germany, in particular European Beech (*Fagus sylvatica*), Sessile oak (*Quercus petraea*),

and other native broadleaf trees; and, less intensive management combined with nature conservation. This increases amounts of deadwood (Doerfler et al 2017) by active and passive measures, comprising harvest remnants such as large tree crowns, fine woody debris, coarse woody debris and naturally developed deadwood e.g. snags or windblown trees. Another aspect of the management strategy is to preserve ten trees per hectare with microhabitats (Kraus *et al.* 2016), including trees with perennial fungi such as *Fomes fomentarius*.

Beetle sample collection

The sampling was conducted in the years 2014 and 2015, year round. During routine forest inspections by team members of the forestry department of Ebrach (in the *managed* areas), and studies by a student intern in the *unmanaged* forest areas, sporocarps of *Fomes fomentarius* were examined for beetle occurrence. In winter especially, the sporocarps are highly visible even up to distances of 50 m and therefore large areas of the forest were surveyed in this way. Areas that were less well covered in 2014 were revisited in 2015 for additional collections. If several fruiting bodies were present three were sampled for beetles. The sampling of beetles was executed only until a height of approximately 2 m. The location of the sampled tree was recorded with the date of the sampling and indicated on a map.

Samples of *Bolitophagus reticulatus* were stored in 99% Ethanol.

Genome analysis and microsatellite development

Microsatellites were designed from genome sequence data, generated from a single beetle individual collected from the northern managed area of the forest. Briefly, DNA was extracted using the salting-out procedure (Sunnucks & Hales 1996). A DNA library was prepared using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England

BioLabs GmbH, Frankfurt am Main, Germany), with NEBNext Multiplex Oligos for Illumina adapters (Index Primer Set 1). Next generation sequencing using the Illumina HiSeq™ 2500 was conducted on a paired-end flow cell with a read length of 100bp according to the manufacturer's instructions (Illumina Inc., San Diego, USA). A *de novo* assembly was performed using the CLC Genomics Workbench (v7.0.4; <http://www.clcbio.com>) under default settings, except using a minimum contig length of 500 bp. For the detection of microsatellites, the software Phobos (v3.3.12; www.ruhr-uni-bochum.de/spezzoo/cm/cm_phobos.htm) was used to identify tri-, tetra-, and penta-nucleotide repeat units. The integrated Primer3 tool in the Unipro UGENE software (version 1.14.0) (Okonechnikov, Golosova & Fursov 2012) was used to pick one primer in each flanking region around the microsatellite. Forty microsatellites, located across multiple contigs to represent the wider genome, were chosen to test for amplification and polymorphism in single-plex using eight beetles sampled across the wider collection area. Primer-dimerization among pairs of primers was also checked in order for an effective multiplex PCR to be developed. Seventeen polymorphic microsatellite primer pairs (Zytynska *et al.* 2018a) were chosen to develop a PCR-multiplex leading to two multiplex combinations with 8-9 primer pairs in each, using three fluorescent dyes: 6-FAM, HEX, and TAMRA, alongside the ROX size standard run on an ABI 3130xl Genetic Analyzer (Applied Biosystems - Life Technologies GmbH, Darmstadt, Germany). Primer concentrations were optimised to ensure that all peaks lay within the 1000-10,000 peak height range for efficient genotyping.

The final PCR multiplex conditions were: 1 µl DNA diluted 1:4, 5 x MyTaq™ Reaction Buffer (Bioline, UK), 2 Units MyTaq™, specific primer mix (Table 1), up to 20 µl with molecular grade water, run at 95 °C for 2 mins, 30 cycles of 95 °C for 15 sec, 60 °C for 15

sec, 72 °C for 15 secs, and then a final step at 72 °C for 2 min. Fragment data was analysed using the software GeneMarker (version 1.75) (Softgenetics LLC, State College, PA, USA).

Data analysis

Unless mentioned otherwise, all statistical analyses were done using R v3.3.0 (R Core Team 2018) in RStudio v0.99.896 (RStudio Team 2018). Basic descriptive molecular statistics at the locus and sampling point levels, were obtained using the package ‘poppr’ in R (Kamvar, Brooks & Grünwald 2015). Using AMOVA (analysis of molecular variance) in Arlequin (v 3.5.2.2), the amount of variation explained by the *a priori* separations of north and south areas of the forest, and the historically managed and unmanaged areas within these was explored.

To assess the effect of isolation by distance across the sample, beetle relatedness from the microsatellite data between all individuals was calculated using ML-relate (Kalinowski, Wagner & Taper 2006). Relatedness measures the probability that two individuals share none, one, or two alleles (gene variants) at a given locus (here, microsatellite), and is averaged across the loci studied giving a range of 0 (unrelated) to 1 (clones or identical twins). These were correlated to geographic distance using a Mantel Test in PaSSAGE (Rosenberg & Anderson 2011). Pairwise combinations of individuals were further grouped by geographic distance into intervals of 2 km, to reveal spatial structuring of the data. A linear model was used to analyse the variation in beetle relatedness across these distance intervals, and post-hoc analysis using a tukey-test identified those distances where beetles were more closely related than others. Factorial simplification from this information was used to reduce the distance intervals, in order to identify spatial scales of relatedness among the sampled beetles. The effect of the different sampling scales (*within-fungi*, *within-tree*, and

among trees) on beetle relatedness (data square-root transformed to fit model assumptions) was analysed using a mixed-effects model, with geographic distance between the individuals as a covariate and tree individual as a random effect (to account for the pairwise structure of the data; resulting residual degrees of freedom were 107, from a total independent sample size of 112 beetles). Factorial simplification was used to test the effect of relatedness between beetles from the same tree (all within-fungi and within-tree samples) compared to those collected on different trees. Data were randomised to check for effects of unequal sample sizes across the sampling scales on the patterns of significance; randomised data showed no significant effects and thus there was no confounding effect of sampling sizes.

To group the beetles into genotype clusters, we used K-means hierarchical clustering, using the package 'poppr' in R. For cluster visualisation, we used the library "adeget" (adegetServer(what = "DAPC"); Jombart (2008)).

Results

Beetle sample collection

We collected 671 individuals of the fungus-inhabiting beetle *Bolitophagus reticulatus* from 390 fruiting bodies of the fungus *Fomes fomentarius*, across 228 trees. Overall, 40 % (91/228) of the recorded trees had more than one fruiting body of *Fomes*, and 50% (195/390) of *Fomes* fruiting bodies were colonized by beetles. A subset of 112 beetles were chosen for genetic analysis, ensuring sufficient replication to analyze genetic relatedness between beetles across the scales and full geographic distribution (Zytyńska *et al.* 2018b). Of these 112 beetles, 51 were collected from the northern area (22 in managed areas and 29 in unmanaged areas), and 61 from the southern area (24 in managed areas and 37 in unmanaged areas). The sampling represented beetles from 97 different fungi on 60 different trees

(*among-trees*); 42 beetles across 14 trees (1 beetle each from 3 individual fungi per tree; *within-tree*); and, 23 beetles across eight trees where 2-3 beetles had been collected from the same fungus (*within-fungus*).

Basic descriptive molecular statistics

Across all individuals and loci (Table 1), the observed Heterozygosity (H_o) was 4.72, expected Heterozygosity (H_e) was 0.702, and evenness 0.80. The separation between the northern and southern regions only explained 6.45% of variation in the molecular data, whereas the difference between *managed* and *unmanaged* areas within these regions explained even less variation (1.08%; Figure 1). The variation across managed and unmanaged areas was only as a result of greater separation in the southern area (Figure 1) as there was slightly higher genetic diversity of beetles within the southern managed ($H_o = 3.61$, $H_e = 0.682$) than the southern unmanaged area ($H_o = 3.18$, $H_e = 0.622$). Such a pattern was not so apparent in the north, yet the genetic diversity of beetles was again slightly higher in the managed ($H_o = 3.37$, $H_e = 0.701$) than the unmanaged areas ($H_o = 3.09$, $H_e = 0.676$).

Beetle population genetic structure

There was a small to moderate effect of geographic distance on beetle relatedness among individuals (Mantel Test: $r = -0.344$, $p < 0.001$), indicating only a low amount of isolation-by-distance. While overall relatedness between individuals was low (0.063 ± 0.003 ; mean $\pm$ SE), relatedness among the beetles was higher between those sampled within an area of 0-2 km (0.157 ± 0.011), and lower between individuals sampled more than 18 km from each other (0.013 ± 0.004 ; Figure 2a); as identified from distance interval analysis. We found that beetles were more genetically related when collected from the same fungus (0.208 ± 0.030) or same tree (0.155 ± 0.021) than those from different trees (0.054 ± 0.003) (sampling scale: $F_{2,107} = 5.37$,

$p=0.006$; Figure 2b). This effect was driven by the difference in relatedness between beetles on the same versus different trees (factorial simplification same fungus or tree vs different trees: $F_{1,108}=8.40$, $p=0.005$), as there was no difference in relatedness of beetles from the same fungus (within-fungi) or from different fungi on the same tree (within-tree) (posthoc: $t=1.77$, $p=0.080$).

The beetles clustered into four main groups (1-4), with one group showing evidence of sub-clustering (4.1 and 4.2; Figure 3). The first cluster was distributed across the whole geographic range, while the others were restricted to certain areas. For example, cluster 2 was only found in the southern part of the sampling area, and cluster 3 in the northern part. Clusters 4.1 and 4.2 spanned both north and south areas (as divided by the main road traversing the sampling area), but were only found in the north-western part of the south site indicating a potential dispersal corridor between the north and south areas. Clusters 1 and 4.2 formed the basal genotype groups of this population, with cluster 2 derived from cluster 1 in the south, and clusters 3 and 4.1 derived from cluster 4.2 in the north (Figure 3).

Discussion

In this study, we sampled the fungivorous beetle *Bolitophagus reticulatus* in areas that were previously uninhabited during a survey in 2004 (Müller 2005b), indicating that this beetle has successfully recolonised these areas over the past ten years. This is likely due to forest management programs that aimed to enrich the amount of deadwood, enabling the spread of the fungus *Fomes fomentarius* on which *B. reticulatus* lives. Using 17 newly-developed microsatellite markers, we found only a weak pattern of isolation-by-distance across the sampling region indicating that the beetles are able to disperse across the landscape. The north-south divide explained only a small proportion of genetic variation (6.45%) while the

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sampling within managed and unmanaged areas explained even less (1.08%). Other studies on different beetle species have found similar levels of variation explained by geographic location and, although it is difficult to compare across different species and systems, similar conclusions were made that this shows weak population structuring (Lagisz *et al.* 2010; Tiroesele *et al.* 2014). While beetle diversity was higher in managed areas, likely due to the larger spatial area from which the beetles were sampled, these differences were very slight and could only explain a significant amount of variation in the southern region. Overall, the relatedness among the beetles sampled was low, with beetles located within 2 km of one another showing higher relatedness than those at distances of 4-16 km, and 18-24 km. While the level of relatedness itself was not high, this does indicate that over the last decade the beetles have readily dispersed distances of up to 4 km but rarely more than 18 km. It is difficult to compare relatedness to those calculated from other species using different molecular markers, yet estimates of relatedness ranging from 0.054-0.208 shows that even beetles from the same fungus are not likely to have the same mother; an individual gets half its genes from the mother and half from the father, so the relatedness from mother to offspring is 0.50, and between full-siblings (with the same mother and father) the average is also 0.50 (an average since, theoretically, siblings can share none, half, or all genes passed on by the parents). By using 17 microsatellites with an expected heterozygosity of 0.702, we limit the errors that would misclassify relationships among beetle pairs (Blouin *et al.* 1996).

The sampled beetles clustered into four main genotypic clusters, with one cluster splitting further into two sub-clusters. This sub-clustering shows that while these beetles are more similar to each other within their main cluster, than to any other main cluster, there is still significant genetic differentiation within it. These two sub-clusters were found in the north-western part of the sampling area, where there is an obvious forest corridor between the

northern and the southern parts of the forest, with one cluster found more to the north-east than the other. Historically, more fungi and beetles had been found in the south and north-western part of the forest, with only young fungal sporocarps, and no beetles, observed in the north-east (Müller 2005a; Müller 2005b; also see Appendix S1). In this newly-colonised area to the north-east, where deadwood was enriched through management schemes for the past ten years, we collected beetles belonging to a genotypic cluster that was not sampled in the south. This genotype is derived from the main cluster that links the north and south part of the forest. Similarly, we also collected beetles belonging to a genotype cluster that was only sampled in the south. This genotype was derived from another genotype cluster that spanned the whole sampling area. Our results suggest that the two basal main genotype clusters are those that span the whole range, particularly the area of forest that connects the north and the south parts. The more isolated clusters are then derived from these and remain in their respective forest areas. This may be due to the abundance of ‘good’ habitat and a chance occurrence that some genotypes dispersed whereas others did not. Alternatively, the more widely-distributed beetle genotypes may differ in dispersal ability or other phenotypic traits enabling more efficient colonisation of new habitats (Jonsson 2003; Niitepõld *et al.* 2009). However, as we see from the isolation-by-distance pattern, it is unlikely that in another ten years we would still detect this isolation and most likely the populations would be even more mixed.

Previous work on the same beetle species suggested that habitat isolation even at small scales of 25-30 m reduced the occurrence of beetles (Rukke & Midtgaard 1998; Sverdrup-Thygeson & Midtgaard 1998). Yet, our results suggest that if the fungus is present then this isolation effect does not have a strong impact on beetle population genetic structure until distances are greater than 2000 m (2 km), or even until 18 km. Indeed, our results show that even after only

ten years of management for deadwood enrichment, the beetles have dispersed into the northern forest area and colonised the sporocarps of the fungi that now grow on the deadwood.

Major lessons

Many forest managers and conservationists nowadays aim to restore deadwood habitats in forest landscapes. Fungi play a prominent role in ecosystems, not only as major decomposers but also by creating suitable habitat for many species feeding on the mycelium or living in the sporocarps (Thorn *et al.* 2015). A recent review of dispersal limitations of saproxylic beetles and their fungi has shown that, in general, fungi experience less dispersal limitation than their insects (Komonen & Müller 2018). Numerous fungal spores can be released in the environment, and while many will land nearby, others can disperse long distances by wind (Dam 2013). In this case, spores only require a suitable deadwood habitat where they land in order to colonise. A lack of certain species of fungi, such as *F. fomentarius*, in the forest, therefore seems to be indicative of a lack of habitat rather than dispersal limitation, at least over the distances documented in the present study. Fungivorous insects, in turn, will be limited to those places where fungal spores have landed and established. However, these insects also have the ability for more active and directed dispersal, and as such are only limited by the availability of the host-fungus within the area and their own life-history traits (e.g. well-developed flight muscles; Jonsson (2003)). *Fomes fomentarius* is a dominant fungus in beech forests, colonizing decaying trees, hence an increase of beech deadwood will result in rapid colonization by this fungus. Small arthropods living in such sporocarps such as *Bolitophagous* seem to be able to track this ephemeral resource successfully over tens of kilometres.

In our study, beetles from almost all genotypes detected in the landscape could be found in the new area to the north, supporting the idea of a broad landscape-wide colonization. Even at the very local scale (within the same tree or fungus) we found low relatedness of beetles suggesting high degree of mixing among individuals from different areas. Such a pattern supports the view that restoration of a forest landscape does not necessarily have to consider the spatial arrangement of deadwood, particularly if a decade is sufficient for representation of most of the genotypes in an area of 30x30 km². Therefore, at least for good dispersers such as *B. reticulatus*, the focus should be more on increasing the amount of dead wood in beech forests, rather than on a particular spatial distribution across the landscape. It remains to be shown whether this pattern holds true for other related beetle species, e.g. *Oplocephala haemorrhoidalis* (Jonsson 2003), which have been found to lack the suite of life-history traits that underlie the dispersal capabilities of *B. reticulatus*. In comparison to *O. haemorrhoidalis*, *B. reticulatus* has better developed flight muscles, a greater 'willingness' to take off and more (but smaller) eggs, which lead *B. reticulatus* to be better suited to colonising patchy habitats such as scattered fruiting bodies in forest fragments (Jonsson 2003). However, even for this rare species, *O. haemorrhoidalis*, a recolonization of previously abandoned areas has been observed in Germany probably due to an increase in *Fomes* (Kohler 2010); a lack of before and after data in this system limits our ability to test this.

For *B. reticulatus*, these results suggest that forest management based around simply increasing deadwood is sufficient to allow recolonization by the fungus, and subsequently the beetle, without an apparent bottleneck in genetic diversity in the beetle population. Indeed, this recolonization was likely benefited by the forest corridor to the north-west indicating that, while sophisticated spatial arrangements of set-aside 'stepping stone' forest patches may not be required, dispersal corridors containing any amount of required habitat (here,

deadwood) will ultimately benefit recolonization of forest fragments. The results further support the view that deadwood enrichment is meaningful also in former intensively-managed stands, in this study as intensively-managed beech but elsewhere this can also refer to plantation-dominated forests, as long as they are embedded in a forest landscape where some relict populations of the fungus and beetle have survived. Further, here we note that habitat availability is important, but recent work also suggests that for a well-functioning forest system we must also consider the diversity of deadwood (Seibold *et al.* 2016). While one type of deadwood may be beneficial for one saproxylic species, other types are used by different species and therefore is an important factor for supporting the natural biodiversity.

Finally we want to make a cautionary note. We are fully aware that the dispersal abilities of deadwood species varies broadly from being flightless to excellent passive or active dispersing organisms. So more research is needed to identify opposing examples to ours and those reported in the literature. However, it seems that fungi that are specialist to specific tree species, or invertebrate species that are highly host-specific to a single fungus species growing on dead wood have to be effective dispersers to track their ephemeral and patchy resource in a landscape; e.g. the pyrophilous Buprestidae *Melanophila acuminata* (Schmitz & Bleckmann 1998), or the aspen colonizing fly *Hammerschmidtia ferruginea* (Rotheray *et al.* 2009). In contrast, flightless invertebrate species are often deadwood generalists with greater habitat choice and, indeed, recent studies in forest landscapes found no support for dispersal-limitation of these flightless saproxylics (Janssen *et al.* 2016). However, there is no question that some dispersal barriers will exist and increase over longer distances and so future work should identify the spatial scales at which these barriers occur.

Conclusions

We show that for successful recolonization of forests by a species of deadwood-dependent fungi and its associated invertebrate there simply needed to be available deadwood habitat, with little restrictions due to dispersal limitation. Indeed, a recent review supports our study in finding that there are few examples of high dispersal limitations in other studies on saproxylic fungi and their insects (Komonen & Müller 2018). The forest scale at which we study this (up to 24 km) is the same scale at which forest managers currently conduct planning and decision-making, and our results indicate that decisions to increase deadwood at this scale can have important effects on local biodiversity (Doerfler *et al.* 2017). Many biodiversity conservation projects focus on improving abundances of rare species that often have specific habitat requirements (Seibold *et al.* 2015b), resulting in an increase in these habitat types. For supporting the general biodiversity in forests, habitat diversity is perhaps the key in forest management to benefit a range of species. In this case, for deadwood species, an increase in both the availability of deadwood, along with increased diversity in the types of deadwood, is needed.

Authors' contributions

JM, ID, WW and MG designed the sampling scheme; ID collected the samples; SZ and SS developed the microsatellites and performed the molecular analysis; SZ analysed the data; SZ, JM, MG wrote the first draft of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

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Data accessibility

Data available via the Dryad Digital Repository. DOI: 10.5061/dryad.pg632hc. (Zytynska et al. 2018b)

Microsatellite primer sequences are deposited in the NCBI Probe database with accession numbers Pr032825846-862: <https://www.ncbi.nlm.nih.gov/probe/?term=Pr032825846> (Zytynska et al. 2018a)

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Table 1. Primer sequences and characteristics of 17 microsatellite loci for *Bolitophagus reticulatus*.

Multiplex/ Locus	Accession Number	Repeat of cloned allele	Fluorescent dye	Primers	Primer mix ratio	Number of alleles	Length of PCR product (bp)	H_E	Evenness
MM1									
FS_BO01	Pr032825846	(CA)26	6-FAM	F: CGGGAGCGACAAGGAAC R: CTACCCTCGCCTCCCCTA	0.04	7	144-162	0.76	0.85
FS_BO02	Pr032825847	(TG)24	6-FAM	F: CGCACCTTTGACACAAAAGA R: TCAAAGCTCCTCTAAAGAGTATTTCC	0.22	11	235-257	0.77	0.73
FS_BO03	Pr032825848	(TGT)14	6-FAM	F: AAGCGGTCAGTCACCCAGT R: TATCCGATCTGAGCGATTCC	0.09	7	314-337	0.71	0.69
FS_BO04	Pr032825849	(AT)25	6-FAM	F: TCGCATTATTTTGACAGTTGAATC R: GACGTGTATCAGTCACGAAAATATG	0.04	5	398-440	0.70	0.85
FS_BO05	Pr032825850	(CAA)12	HEX	F: ACGCAAAGAGTTGGAGGATG R: AGCGTCTTCGCCAAATCC	0.07	5	191-215	0.55	0.72
FS_BO06	Pr032825851	(AC)17	HEX	F: GTTAGCCTCATGTAAGCAGGTCA R: CTACATCATCCCGAAATCAGC	0.22	3	278-282	0.41	0.64
FS_BO07	Pr032825852	(ATT)20	HEX	F: CTTGGTTTCACCACCAAATC R: TGAGCATTTGGATCATCAGG	0.11	6	311-357	0.60	0.64
FS_BO08	Pr032825853	(AT)20	TAMRA	F: ACAGTTCATTATAAGGCCAGATCG R: AACTTTGTTGATGGCACTTCC	0.22	8	317-346	0.75	0.79
MM2									
FS_BO09	Pr032825854	(AGTC)9	6-FAM	F: CGTTAGCTCTTACGGCTTGG R: GAACGTGTATTCTTTGGGTGAAC	0.06	3	174-204	0.47	0.68
FS_BO10	Pr032825855	(CA)20	6-FAM	F: CGGCTTTTAAATTGCTGCTC R: TCGGCCATAATCTCCAA	0.09	12	255-283	0.82	0.77
FS_BO11	Pr032825856	(GT)19	6-FAM	F: AAATTTCGGCACAAAATCCTG R: GTCCATCCATTCTTTTCCTGT	0.09	8	320-336	0.83	0.85
FS_BO12	Pr032825857	(TG)20	6-FAM	F: CGATAAACCCCGAGAAAAGAG R: CCCGCCAAAAATTATTAATCG	0.15	6	433-463	0.80	0.92
FS_BO13	Pr032825858	(TAA)14	HEX	F: ACTTCGACAGGCGTTTACAG R: CGAAATCGTGAATCGGTAGC	0.11	8	186-214	0.80	0.83

FS_BO14	Pr032825859	(TAT)11	HEX	F: CGTGGGGAGTACACTGTTGC R: CCTTTATGACGTTTCAGAGGACTG	0.11	5	294-306	0.64	0.86
FS_BO15	Pr032825860	(GT)16	HEX	F: AATCGTCGCCGATGTTTAAG R: GCCCGGTATAAATTACAGGATG	0.09	7	337-349	0.82	0.94
FS_BO16	Pr032825861	(AAT)10	TAMRA	F: TGAAGCACATTGCCAAAATC R: GGGTGCAAGACCTCGTTAAT	0.20	6	205-230	0.78	0.82
FS_BO17	Pr032825862	(TATT)9	TAMRA	F: GTCGCGCAAATCAACAGTAA R: GCAAAAGTTTACGGAGTGAGACA	0.09	4	358-374	0.71	0.90

Accession numbers for the NCBI Probe database (Zytynska *et al.* 2018a). Diversity: H_E , expected heterozygosity. Primer mix ratio shows the relative volumes of primers

used in each mix.

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Figure 1. Beetle genetic analysis indicates a separation of the population between the north and south areas of the forest. Each dot represents one beetle, and groups were selected *a priori* due to the collection location (north or south) and the historical management practice (managed or unmanaged). Beetles from the south also exhibit higher genetic diversity than the north, with some separation between the managed and unmanaged areas. Inset shows the DA eigenvalues from the DAPC analysis, indicating the stronger separation on the first axis (horizontal: North vs South) then the second axis (vertical: Managed vs Unmanaged).

Figure 2. Relatedness of all individual beetles across the geographical area and sampling scales. (a) Beetles collected from the same tree (0 km) were more related than those located at a greater distance from one another, yet the relatedness values are still very low even at small spatial scales. Vertical lines denote the separation of three spatial scales identified in the analyses (0-2, 4-16, 18-24 km). Points show the mean values and the grey band shows the standard error around each data point. (b) Beetles collected within the same fungus or tree were more related to each other than those collected on different trees ($F_{1,108}=8.40$, $P=0.005$). Violin plots show a combination of a box plot and a kernel density plot; the white dot in the middle shows the median value, with interquartile range as the black rectangle, and the 95% confidence interval around the median as the vertical line. The grey area shows the frequency of values across the values of relatedness.

Figure 3. *Bolitophagus reticulatus* beetle population genetic structure in the Steigerwald Forest, Germany. The map shows the distribution of the sampled beetles: each coloured shape represents one genotyped beetle (those collected on the same tree are overlaid), the colour and shape depicts the genotype cluster to which it belongs. The black dashed line

separates the areas that were previously uninhabited (extinction) by the beetle and the areas in the south that were inhabited (survival). The relationship between genetic clusters is shown on the top right, where the minimum spanning tree (black lines) links the most similar genetic clusters.