Nests of red wood ants (*Formica rufa*-group) are positively associated with tectonic faults: a double-blind test

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Ecological studies often are subjected to unintentional biases, suggesting that improved research designs for hypothesis testing should be used. Double-blind ecological studies are rare but necessary to minimize sampling biases and omission errors, and improve the reliability of research. We used a double-blind design to evaluate associations between nests of red wood ants (*Formica rufa*, RWA) and the distribution of tectonic faults. We randomly sampled two regions in western Denmark to map the spatial distribution of RWA nests. We then calculated nest proximity to the nearest active tectonic faults. Red wood ant nests were eight times more likely to be found within 60 meters of known tectonic faults than were random points in the same region but without nests. This pattern paralleled the directionality of the fault system, with NNE-SSW faults having the strongest associations with RWA nests. The nest locations were collected without knowledge of the spatial distribution of active faults thus we are confident that the results are neither biased nor artefactual. This example highlights the benefits of double-blind designs in reducing sampling biases, testing controversial hypotheses, and increasing the reliability of the conclusions of research.

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17

18   **Abstract**

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20   designs for hypothesis testing should be used. Double-blind ecological studies are rare but

21   necessary to minimize sampling biases and omission errors, and improve the reliability of

22   research. We used a double-blind design to evaluate associations between nests of red wood ants

23   (*Formica rufa,* RWA) and the distribution of tectonic faults. We randomly sampled two regions

24   in western Denmark to map the spatial distribution of RWA nests. We then calculated nest

25   proximity to the nearest active tectonic faults. Red wood ant nests were eight times more likely

26   to be found within 60 meters of known tectonic faults than were random points in the same

27   region but without nests. This pattern paralleled the directionality of the fault system, with NNE-

28   SSW faults having the strongest associations with RWA nests. The nest locations were collected

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31   designs in reducing sampling biases, testing controversial hypotheses, and increasing the

32   reliability of the conclusions of research.

33

34   **Introduction**

35   A central question for ecology—the study of the distribution and abundance of organisms—is

36   why do organisms occur where they do? Explanations include relationships between organisms

37   and specific environments, interspecific interactions, or random chance. All of these explanations

38   have been suggested to apply to ants, one of the most widespread and abundant taxon on Earth

39   (Hölldobler & Wilson, 1990; Lach, Parr & Abbott, 2010). Berberich and Schreiber (2013) and

40   Berberich et al. (2016b) reported a seemingly peculiar positive spatial association between the

41   geographically widespread, conspicuous red wood ants (*Formica rufa*-group) and seismically

42   active, degassing tectonic faults. This work has been difficult to publish because reviewers have

43   suggested that the authors are ignoring alternative explanations or are ignorant of the basic

44   biology of ants. Such critiques are familiar to anyone who has proposed a new or controversial

45   hypothesis, but it is indeed peculiar that ants would be associated with degassing tectonic faults.

46   Here, we confront the observations of Berberich and colleagues using a double-blind

47 study. Double-blind studies, in which treatment assignments (or data collected) are concealed to

48 researchers and subjects, are the most robust ones for testing any hypothesis, especially

49 controversial ones, and increase the reliability of results and conclusions (Holman et al., 2015).

50 Double-blind designs are routine in medical sciences, but rare in ecology (Kardish et al., 2015).

51 To test more robustly the hypothesis that RWA nests are associated with active faults, we used a

52 double-blind design in which myrmecologists who were unaware of this hypothesis or any

53 published work on links between RWA and seismic activity (IDT and RRR) were sent into the

54 field to map RWA nests. Simultaneously, maps of active tectonic faults in the region were

55 obtained and organized by geoscientists (GMB and MBB) without any knowledge of the field

56 data. With these two independently collected datasets, we then asked whether ants were

57 positively associated with tectonic faults.

58

59 **Methods and Materials**

60

61 *Sampling design and data collection*

62 With no prior knowledge, IDT and RRR surveyed two regions of the Jutland Peninsula of

63 Denmark: Thisted in the north and Klosterhede in the south (Fig 1A-C). Both study areas are

64 located within the Permian–Cenozoic Danish Basin, which was formed by crustal extension,

65 subsidence, and local faulting (Nielsen, 2003). This basin is bounded in the north by the

66 seismically active, NW-SE striking fault system of the Sorgenfrei-Tornquist-Zone (STZ in Fig.

67 1A) and in the south by the basement blocks of the Ringkøbing–Fyn High and the Brande

68 Graben (RFH and BG in Fig. 1A). The dominating compressional stress field is orientated

69 primarily NW-SE direction (Fig. 1D) but scatters in different regions (Gregersen, 2002;

70 Helmholtz Centre Potsdam - GFZ German Research Centre for Geosciences, 2008).

71   The Thisted region (~670 km^2) included parts of the Thy National Park. The Klosterhede

72 region (~700 km^2), included the Klosterhede plantation, the third largest forested area in

73 Denmark. Landscapes and vegetation communities varied between the two sampling regions.

74 Coastal dunes dominated the Thisted region, whereas a mix of grasslands, pine and oak forests,

75 and conifer plantations dominated the Klosterhede region. Agricultural lands in both regions

76 were primarily rapeseed plantations.

77   Before surveying for RWA nests, and with no prior knowledge of the spatial distribution

78 of tectonic faults, the two regions were subdivided into ~1000, 1000 × 1500-m grid cells. One

79 hundred of the cells in each region were selected at random for mapping RWA nests, using the

80 rnorm function in R (version 3.31). At each site, we used an adaptive sampling design to search

81 for RWA nests. If no RWA nests were encountered within an initial 30-minute sampling period,

82 we considered RWA to be absent from the grid cell. However, if a RWA nest was encountered

83 within an initial 30 minutes of searching, the survey was continued for an additional 30 minutes;

84 this process was repeated until no new nests were found within the survey grid cell boundaries.

85 The location of each RWA nest found was recorded using a Garmin Oregon 600 GPS unit

86 (Garmin Olathe, Kansas, USA); three individual worker ants were collected for subsequent

87 species identification. Voucher specimens were deposited in the Natural History Museum of

88 Denmark, Copenhagen.

89   GMB and MBB synthesized published data on geotectonic structures of the two study

90 areas (data in Supplementary Online Material) with tectonic maps provided by Stig Pedersen

91 (Geological Survey of Denmark) and the GEUS Map Server (The Geological Survey of

92 Denmark and Greenland, 2015); they did so with no knowledge of the distributions of the RWA

93 nest data collected by IDT and RRR.

94

95 *Spatial data and analyses*

96 Spatial clustering of RWA nests was examined with Ripley's *K* (Ripley, 1977). The distance

97 from each nest to the nearest fault line was calculated using the "distmap" function in the

98 "spstat" library (Baddeley, Rubak & Turner, 2015) using R (version 3.3.1) (R Development Core

99 Team, 2014). We then estimated ρ: the effect of the spatial covariate (*i.e.,* distance to faults) on

100 the spatial intensity of the locations of the ant nests and the locations of cells without ants

101 (Baddeley et al., 2012). Finally, we used a Komlogrov-Smirnov (K-S) test to test if observed

102 RWA nests were closer to faults than locations sampled (i.e. the center of the sampled grid cell)

103 where no RWA nests were detected. We attempted to reduce sampling bias resulting from spatial

104 autocorrelation by using a random sampling grid. Remaining spatial autocorrelation or clustering

105 most likely is related to the polydomous and polygynous colony structure of many *F. rufa*-group

106 species (Seifert, 2007)

107

108 **Results and Discussion**

109

110 *RWA nests occur closer to fault lines than expected by chance*

111 RWA nests occurred in 28 of the 200 random grid cells (12 in the Thisted region and 16 in the

112 Klosterhede region). When RWA ants occurred in a sampled grid cell, there were generally > 1

113 nest; in total we detected 273 nests of *Formica* species. All but four (one *F. serviformica* and

114 three *F. fusca*) were nests of *Formica rufa*-group ants: 86% were nests of *F. polyctena* and 12%

115 were *F. rufa*. In both regions, RWA nests were spatially clustered according to Ripley's *K*, but

116 cells without ants were not spatially clustered (Fig. 1).

117 Covariance of RWA nests and faults was highest within 60 m of faults (Fig. 2A), and

118 approached zero at greater distances. In contrast, there was no observable covariance between

119 cells without RWA nests and their distance from faults (Fig. 2B). RWA nests were

120 approximately eight times more likely to be found at distances <60m from a fault than were cells

121 without ants (*K-S* test D= 0.373, *P* <0.001).

122 The directionality of a fault also affected the covariance between the spatial intensity of

123 RWA nests and their distance to faults (Fig. 2C–2H). Specifically, at distances < 100 m from an

124 active fault and relative to grid cells lacking ants, RWA nests were 10 times more likely along

125 faults trending NNE–SSW (Fig. 2F) and up to eight times more likely on faults trending NW–SE

126 or NNW–SSE (Fig. 2C, 2D). These directions are associated with the present-day main tectonic

127 stress field and its scattering directions (Helmholtz Centre Potsdam - GFZ German Research

128 Centre for Geosciences, 2008). In contrast, RWA ants were only 2–4 times more likely to faults

129 trending NE–SW or WNW–ESE (Fig. 2E, 2G), and did not occur adjacent to faults trending

130 ENE–WSW (Fig. 2H).

131 To address further whether polydomous colony structure of *F. polyctena* could have

132 skewed our results, we repeated the analysis for *F. rufa* alone. Similar to the overall pattern,

133 *Formica rufa* tended to be clustered within 30 meters from existing degassing faults (Fig 3A).

134 We also tested this pattern without considering individual nests but only the plots where RWA

135 was present; once again the pattern remained consistent and showed a peak in association with

136 faults at distance between 0 and 30 meters (Fig. 3B). This suite of evidence leads us to propose a

137 hypothesis that drives the observed biological pattern and should be tested in subsequent studies.

138 Previous work has associated microhabitat availability to the spatial structure of ant colonies

139 (Scharf, Fischer-Blass & Foitzik, 2011). In this study system, we argue that the underlying fault

140 lines may be providing a warmer thermal microhabitat, which drives the spatial clustering of

141 RWA colonies.

142 *On the use of double-blind studies in ecology*

143 The scientific method emphasizes accurate, unbiased, and objective experiments or observations.

144 Because research results can be biased by design or our underlying belief in the correctness of

145 our hypothesis (confirmation bias: (Nickerson, 1998)), repeatable results and reliable conclusions

146 require that investigators do as much as possible to minimize bias in all aspects of a research

147 project (e.g., Rosenthal & Rosnow, 2007). Double-blind designs provide the gold-standard for

148 unbiased experiments (Holman et al., 2015).

149   In the interest of avoiding bias and increasing the repeatability and reliability of

150 ecological research, we suggest that the benefits of double-blind studies far outweigh the

151 additional costs and logistical complications of creating blinded research teams. The need for

152 multiple research teams leads directly to increased costs and additional project coordination.

153 Trade-offs among personnel, sampling effort, and sampling intensity depend on available

154 resources. In our study, for example, we reduced sampling effort by randomly, not exhaustively,

155 sampling the ~1400 km^2 of the pre-defined study regions. A second cost of a double-blind study

156 such as ones focused on species occurrences is the general tendency to focus on where a species

157 occurs, as opposed to where it does not. For example, most species distribution models are based

158 only on "presence-only" data, as absences are rarely recorded (Berberich et al., 2016a).Yet as we

159 have shown here, the samples of locations lacking RWA nests were crucial for determining

160 whether RWA nests and fault systems had meaningful patterns of covariance.

161   Double-blind experiments remain rare in ecology (Holman et al., 2015) but their

162 importance cannot be overestimated. Results and conclusions of double-blind studies are

163 unlikely to be biased by the views and perspectives of the researchers themselves. Investment in

164 appropriately replicated double-blind studies also may be more cost-effective because they rarely

165 need to be repeated, even if the results are unexpected. Just as double-blind studies in medicine

166 have led to reliable treatments that for injury and disease, double-blind studies in ecology will

167 provide us with high-quality unbiased data of how the natural world is structured and is

168 changing.

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214

Figure 1

Figure 1:

A) Map of Jutland Peninsula, highlighting the two sampling regions and major tectonic units . Thisted (Figure 1B) and Klosterhede Regions (Figure 1C) are shown in detail. Thisted region (North) and the Klosterhede region (South) are shaded in red. Blue points indicate absence of RWA from grid survey; red points indicate the location of RWA nests. D) Distribution of faults in the Jutland Peninsula (after Veijbeck, 1997; Petersen et al. 2008; Pedersen & Gravesen 2010 and GEUS Server, 2015) with red and blue points as in C and D.
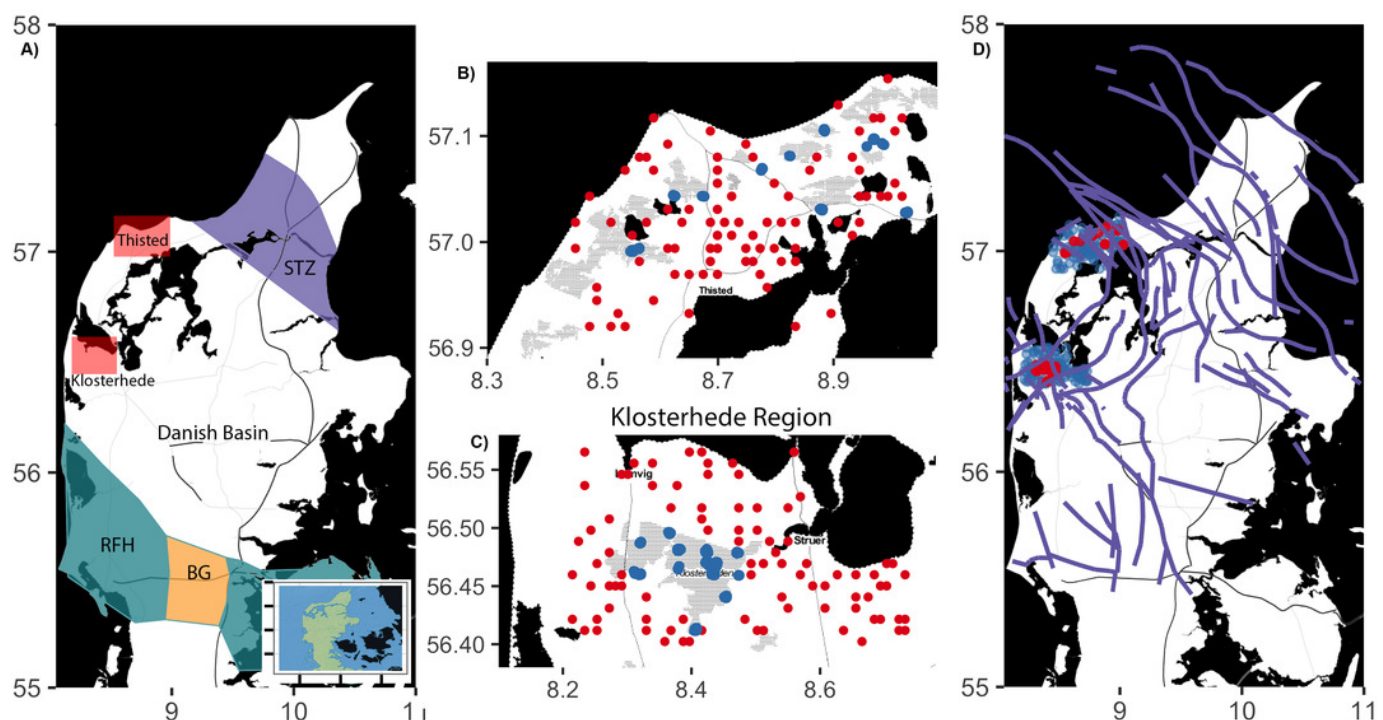
Figure 2

Figure 2:

A) Correlation of ant distributions with tectonic fault zones in the region. B) Correlation of ant absences with tectonic fault zones in the region. C-H) Correlations of ant distributions with direction patterns of tectonic fault zones in the pooled dataset.
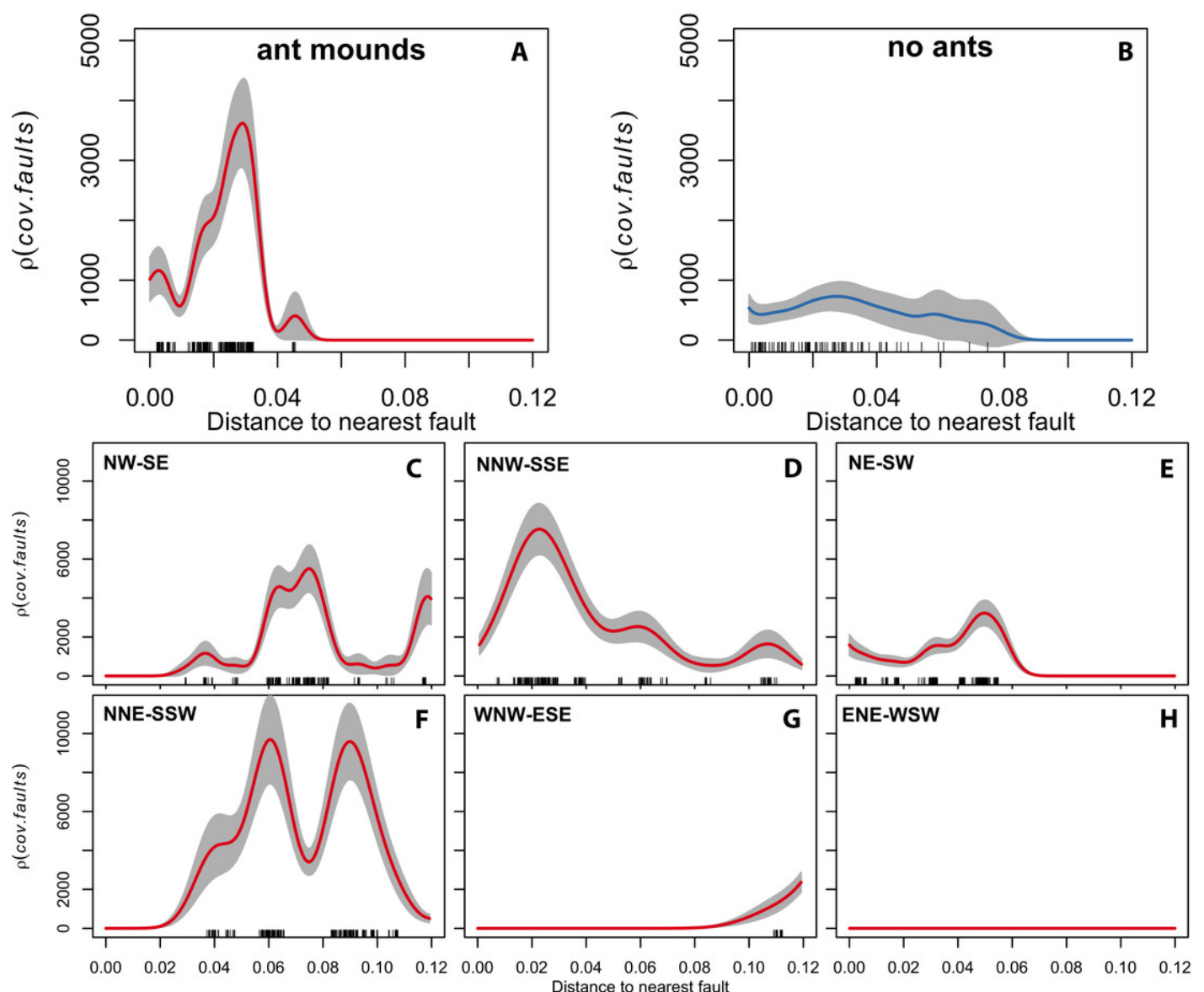
Figure 3

Figure 3:

A) Spatial correlation of *Formica rufa* with tectonic faults B) Spatial Correlation of grid units with RWA with tectonic faults.