Are bacterial endosymbionts reproductive manipulators in the bark beetle *Ips acuminatus*?

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Abstract: Der Borkenkäfer *Ips acuminatus* (Gyllenhal 1827) (Coleoptera: Curculionidae: Scolytinae) zeichnet sich durch eine interessante Fortpflanzungsbiologie aus. Neben bisexueller Reproduktion gibt es auch Populationen, die einen starken Weibchen-Überhang aufweisen und sich parthenogenetisch vermehren. Frühere Studien zeigten, dass dies mit einer Triploidie der Weibchen zusammenhängt. Hier präsentieren wir Daten zur Infektion von *I. acuminatus* mit vererbbaren, bakteriellen Endosymbionten, die das Reproduktionsverhalten von Arthropoden zugunsten von Weibchen verschieben können, um abzuschätzen, ob diese die Parthenogenese von *I. acuminatus* beeinflussen oder gar verstärken können. Hierzu führten wir ein PCR-Screening mit spezifischen Primern für *Cardinium*, *Rickettsia*, *Spiroplasma* und *Wolbachia* durch – vier endosymbiontische Bakterien, die die Fortpflanzung vieler Arthropoden verändern können. Unsere Ergebnisse zeigen keine Endosymbionten-Infektion von *I. acuminatus* und somit keinen potentiellen Effekt auf dessen Fortpflanzungsbiologie. Wir diskutieren alternative Erklärungen für parthenogenetische Reproduktion bei *I. acuminatus* und vergleichen unsere Daten mit anderen Curculioniden-Spezies.

Key Words: endosymbionts, reproductive manipulators, parthenogenesis, pseudogamy, *Wolbachia*, *Cardinium*, *Spiroplasma*, *Rickettsia*

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Introduction

The pine bark beetle *Ips acuminatus* (Gyllenhal 1827) (Coleoptera: Curculionidae: Scolytinae) occurs in wide parts of Eurasia where it infests various tree species of the genus *Pinus* (Pfeffer 1995, Cognato 2015). In Europe, it mainly utilizes Scots pine, *Pinus sylvestris*, and Austrian pine, *Pinus nigra*. This beetle can be a forest pest as it attacks stressed pines and transmits blue-stain fungi (Postner 1974). Moreover, *I. acuminatus* expresses a remarkable reproductive behavior: In addition to bisexual reproduction – where one male individual mates with usually three to five females in one mating chamber (Postner 1974) – a specific mode of parthenogenesis has been described. In certain European populations female-biased broods were found which reproduce via pseudogamy. This means that females produce clonal offspring, however, they need sperm for successful fertilization although genetic material from the male is not inherited to the offspring (Bakke 1968, Kirkendall 1990, Kirkendall & Stenseth 1990, Løyning 2000, Meirmans & al. 2006). Although triploidy in females was found to be involved in parthenogenesis (Lanier & Kirkendall 1986), other factors could cause or reinforce pseudogamy in *I. acuminatus* as well. For instance, maternally inherited bacterial endosymbionts could yield in reproductive alterations of this bark beetle. These bacteria are found in a broad range of terrestrial arthropod species (Weinert & al. 2015). They are usually transmitted vertically – from infected female hosts to their offspring – although inter- and intraspecific horizontal transfer can occur as well (Schuler & al. 2016). Bacterial endosymbionts are of particular interest because they can alter the reproductive outcome of infected hosts

by expressing reproductive phenotypes, like parthenogenesis, cytoplasmic incompatibility, feminization or male-killing (Engelstädter & Hurst 2009). Common endosymbiotic bacteria in terrestrial arthropods are *Wolbachia*, *Cardinium*, *Rickettsia* and *Spiroplasma* (Weinert & al. 2015).

Here, we performed a study to get an insight in the endosymbiont community of *I. acuminatus* in order to assess a potential influence of these bacteria on the reproductive biology of this bark beetle species.

Material and Methods

Samples and DNA extraction

Ips acuminatus individuals were collected from Scots pine near Brixen, South Tyrol, Italy. Beetles were subsequently reared under laboratory conditions at 20 °C and long-day conditions (L : D 16 : 8) in logs of *P. sylvestris*. Twenty-four males and 24 females were used to analyze their endosymbiont infection status.

Endosymbiont screenings

PCR screenings for the presence of *Cardinium*, *Rickettsia*, *Spiroplasma* and *Wolbachia* were performed with taxon-specific primers (PCR conditions and further details see Schebeck & al. 2018). *Cardinium* screenings were done using the primer pair CLO-F1/CLO-R1 (Weeks & al. 2003) with *Cardinium*-infected *Bemisia tabaci* (Gennadius 1889) as positive control. *Rickettsia* infections were studied with the forward primer 528F and the reverse primer 1044R (Chiel & al. 2009), also using *Rickettsia*-harboring *B. tabaci* as positive control. To detect *Spiroplasma* in *I. acuminatus* the primers SpixoF and SpixoR were used (Duron & al. 2008). We used *Dermanyssus gallinae* (De Geer 1778) mites infected with *Spiroplasma* as positive control. *Wolbachia* infections were studied by applying the forward primer 81F and the reverse primer 891R (Braig & al. 1998, Zhou & al. 1998) with *Wolbachia*-infected *Rhagoletis cerasi* (Linnaeus 1758) flies as positive control.

PCR products were separated by gel-electrophoresis using a 2 % agarose gel with GelRed nucleic acid dye (Biotum, Hayward, CA, USA).

Results

Our PCR screening for *Cardinium*, *Rickettsia*, *Spiroplasma* and *Wolbachia* in the bark beetle *I. acuminatus* did not reveal an endosymbiont infection with none of the four bacteria. By applying electrophoretic separation of PCR products, no positive banding pattern of *I. acuminatus* samples was found.

Discussion

Our results of a screening for the presence of the bacteria *Cardinium*, *Rickettsia*, *Spiroplasma* and *Wolbachia* do not suggest an endosymbiont infection of *I. acuminatus*. Hence, we cannot draw the conclusion that endosymbionts cause or reinforce the pseudogamous reproduction of this bark beetle.

Pseudogamy in bark beetles of the genus *Ips* is a rare phenomenon, with at least two evolutionary trajectories. It has been described from a few North American species, for example, in *Ips tridens* (Mannerheim 1852) or *Ips borealis* Swaine 1911, and only in one European species, *I. acuminatus* (Lanier & Kirkendall 1986). The main driver of this reproductive mode seems to be a triploidy (3n = 48) in female beetles, whereas bisexual males and females are diploid (2n = 32) (Lanier & Kirkendall 1986).

Intraspecific differences in the reproductive biology as an effect of an endosymbiont infection were reported from another curculionid species. The weevil *Pantomorus postfasciatus* (Hustache 1947) exhibits populations that reproduce parthenogenetically – and are infected with *Wolbachia* – and other populations that have bisexual reproduction and do not harbor this endosymbiont (Elias-Costa & al. 2019). A broad-scale study including various populations with different reproductive modes of *I. acuminatus* could shed more light on an endosymbiotic influence on its pseudogamous behavior. This might provide a comprehensive picture of its reproductive biology and underlying mechanisms of parthenogenesis.

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