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Investigating Social Immunity in Swarming Locusts via a Triple Animal–Robot–Pathogen Hybrid Interaction

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Social immunity involves collective defensive strategies against infectious diseases. Despite its prevalence in eusocial insects, little is known about social immunity in non-eusocial organisms like gregarious locusts. To address this gap, an emergent biohybrid approach bridging robotics and ethology is employed to study the behavior of the gregarious phase of *Schistocerca gregaria* in response to the entomopathogenic fungus *Beauveria bassiana*. Herein, the first animal–robot–microorganism interaction is developed to explore how infected biomimetic agents (IB) influence healthy locust behavior compared to healthy biomimetic agents (HB), as well as to infected and healthy non-biomimetic controls (INB, HNB). Significant differences in locust responses to different agents, including latency duration, grooming behavior, tactile interactions, and aggression are observed. In healthy locusts, the increased grooming and tactile interactions in response to IB highlight potential preventive measures against pathogen transmission. Also, tactile interaction behavior is notably extended toward IB, emphasizing the role of reciprocal hygiene in limiting pathogens spread within the swarm. Infected locusts exhibit altered behaviors, including increased interaction with any robotic agents, potentially to be cleaned of fungal conidia. This animal–robot interaction study reveals social immunity dynamics in non-eusocial organisms, with implications for pest control, evolutionary ecology, social complex systems, and bioinspired engineering design.

1. Introduction

Collective defensive strategies to counteract infectious diseases have been documented in many social insects such as bees, ants, wasps, and termites.[1–4] These behavioral and physiological defense measures produce the emergence of a complex phenomenon known as social immunity.[4,5] Just to list a few examples, honeybees can recognize cuticular hydrocarbons associated to sick individuals or volatiles emitted by infected brood to remove them from the hive.[6,7] Also, they can adopt social distancing in response to parasitic invasions.[8] In several ant species, workers prevent disease by removing parasite materials from the pupae, and kill the already infected ones by identifying changes in cuticular hydrocarbons;[9] they also display aggressive behaviors toward infected nestmates.[10] Wasps release long-lasting antiseptic substances in their nest also protecting the following generation.[11] Termites perform allogrooming toward nestmates exposed to pathogens and switch to cannibalism at later stage of infection.[12–14]

Several definitions of social immunity initially referred only to those strategies against pathogens and parasites performed by eusocial species.[15,16] Later, this concept has been also extended to anti-parasite defenses implemented by any other group-living species, beyond eusociality.[17]

Research on group-living species, including fish, offers additional perspectives on the dynamics of pathogen transmission and collective defenses. For instance, parasite infections have been shown to disrupt escape wave propagation in fish shoals, increasing predation risks for both infected and uninfected members.[18] Similarly, fish have demonstrated the capacity to engage in social distancing behaviors, avoiding robotic conspecifics displaying morphological or locomotion anomalies that mimic disease symptoms.[19] Furthermore, parasitic infections have been found to impair movement abilities in fish, altering group cohesion and leadership dynamics.[20] These studies underscore how infections can influence collective behaviors across taxa, providing valuable insights to hypothesize about the broader mechanisms driving social immunity and informing the current investigation into locust swarms.

Locusts are phase polyphenic insects that transit from a solitary phase to a gregarious one depending on their population density.[21] Locusts' gregarious habits, consisting in the attraction to other conspecifics and formation of large swarms, constantly expose individuals to dense-crowed conditions particularly favorable to pathogen and parasite transmission.[22,23] To counteract infections at individual level, locusts have been reported to raise

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their body temperature above the normal set point to reduce pathogen virulence.[24,25] Being ectotherms, this thermoregulating process has behavioral bases in locusts (e.g., behavioral fever) that can chose warmer habitats, bask in the solar radiation, or increase motor activity to set their body temperature to detrimental levels for the development of pathogens.[25,26] In some Orthoptera, behavioral fever has been reported to be selective for specific pathogens and parasites.[27] Interestingly, gregarious locusts resulted more resistant to entomopathogenic fungi than locusts in their solitary phase, benefiting from "density-dependent prophylaxis".[28]

However, little is known about the effect of pathogen infections on social interactions among locust swarm members and the group-level behavioral strategies that emerge in response to microbial diseases. Herein, we examined several behaviors in gregarious locusts that are critical for understanding their social dynamics and responses to infection. Specifically, we measured 1) latency, defined as the time taken to initiate any motor display after the experiment began, which may reflect the individual's engagement with or avoidance of conspecifics or agents; 2) spatial preferences, assessing the time spent by individuals in different sections of the experimental arena, which indicates attraction or repulsion toward agents; 3) grooming duration, encompassing self-cleaning behaviors often linked to pathogen defense; 4) tactile interactions, involving physical contact with the agents, such as leaning on them or engaging in cleaning their surfaces; and 5) aggressive acts, such as kicks directed at the agents, which may signal a form of pathogen-avoidance aggression.

In this study, we proposed a bionic approach based on the animal–robot interaction paradigm to test how pathogen infections influence social interactions and group-level behavioral strategies in locust swarms. We selected the gregarious phase of *Schistocerca gregaria* Forskål (Orthoptera: Acrididae) as a model organism to investigate whether an "infected" biomimetic agent (IB), inoculated with the entomopathogenic fungus *Beauveria bassiana,* evoked behavioral changes in healthy locust individuals compared to their responses to a similar artificial agent not inoculated, named the "healthy biomimetic agent" (HB). The "infected" or "healthy" status of the agents was defined by the presence or absence of the pathogen inoculation, meaning that the primary characteristic distinguishing IB from HB was olfactory, due to the pathogen's chemical signals. This approach allowed us to mimic the potential olfactory cues of an infected individual. Morphological differences in the artificial agents, such as shape and color, were standardized across all biomimetic agents to ensure their acceptance by real locusts and were not associated with the presence or absence of the pathogen. Additionally, we tested how infected locusts behaved with the biomimetic agents, building a comprehensive picture of the social interactions of both healthy and infected individuals. To further investigate the role of agent appearance in social interactions, we exposed locusts to infected non-biomimetic agents (INB), such as a white object with an elliptical silhouette of the same size as the biomimetic agent, and healthy non-biomimetic agents (HNB).

The animal–robot interaction approach represents a novel interdisciplinary field bridging engineering and ethology, advancing the understanding of animal behavior and evolutionary ecology.[29–31] This method offers advantages over traditional approaches, such as the controllability of biomimetic agents and increased standardization of bioassays.[32–36] For instance, dummies operated by humans have enhanced interactions with various insect species, providing improved biomimicry and detectability.[37] Robotic artifacts further enhance lifelike stimuli compared to methods like dummies, video playbacks, or mirrors, enabling more realistic and sustained interactions.[38–42] Additionally, biomimetic robots align with the "3 Rs Principle," promoting animal welfare during experiments.[43,44]

With this pioneer research, we hypothesized that pathogen infection would elicit changes in the critical behaviors studied. For example, we expected that grooming might increase in response to the presence of an infected agent as a potential attempt to remove contamination, while tactile interactions and latency could reflect avoidance of infected agents. Aggressive behaviors might similarly serve as a distancing mechanism to minimize contact with perceived threats. Our investigation integrates these hypotheses with a comprehensive biohybrid approach to explore the complex social dynamics of healthy and infected locusts, shedding light on potential parallels with social immunity observed in other group-living organisms.

2. Results

To investigate the effects of pathogen infections on locust social interactions, we employed a biohybrid experimental setup combining biomimetic and non-biomimetic robotic agents with healthy and infected *S. gregaria*. Biomimetic agents, resembling adult locusts in shape and coloration, were 3D-printed and painted with nontoxic pigments. These agents followed a precise elliptical path, magnetically controlled by an external robotic platform. Agents were inoculated with either *B. bassiana* to simulate infection or sterile water as a control. The arena, divided into robot, exploration, and release sections, allowed measurement of key behaviors, including latency, spatial preferences, grooming, tactile interactions, and aggression (**Figure 1**A–F).

2.1. Impact of Different Robotic Cues on Healthy Locusts' Behavioral Response

In this experiment, we investigated if the inoculation of the entomopathogen on different artificial agents triggered some form of behavioral strategies in healthy *S. gregaria* individuals.

The time locusts spent motionless before doing any motor display (e.g., latency), once the experiment started, was significantly affected by different artificial agents ($\chi^2 = 86.12$, $d.f. = 3$, $P < 0.0001$). Latency duration was longer when locusts were exposed to HB compared to IF ($Z = -6.09$; $P < 0.0001$) and INB ($Z = -5.93$; $P < 0.0001$). Latency duration was longer when locusts were exposed to HNB compared to IB ($Z = -6.09$; $P < 0.0001$) and INB ($Z = -5.93$; $P < 0.0001$) (**Figure 2**A).

The time spent by *S. gregaria* individuals in the robot section was significantly affected by different artificial agents ($\chi^2 = 181$, $d.f. = 3$, $P < 0.0001$). Locusts spent significantly more time in the robot section when HB was exposed than when IB ($Z = -8.61$; $P < 0.0001$), HNB ($Z = -8.54$; $P < 0.0001$), and INB ($Z = -8.67$; $P < 0.0001$) were exposed. The time spent in the robot section was significantly longer with HNB than with IB ($Z = -8.67$;

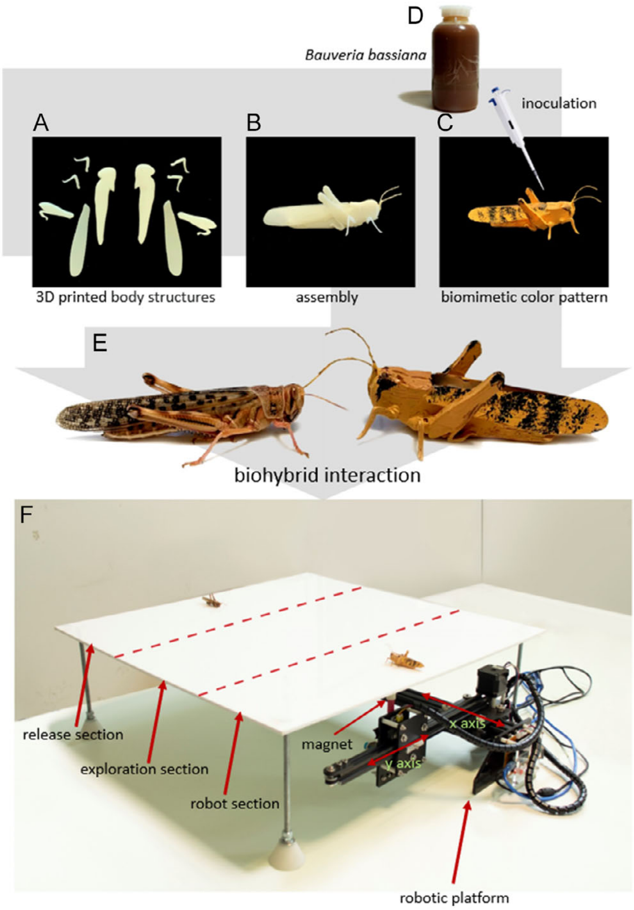

Figure 1. Representation of the biomimetic design process and experimental procedures to establish the biohybrid interaction between the biomimetic agent and *Schistocerca gregaria*. A) Morphometric traits of locusts were independently fast prototyped, B) subsequently assembled and C) painted to visually resemble the integumental color pattern of gregarious *S. gregaria*. D) Robotic cues were dorsally inoculated with 2 μL of the commercial *Bauveria bassiana* bioinsecticide, or with an equal amount of sterile distilled water as control. E) Antennal palpation by a locust individual during a hybrid tactile interaction with the biomimetic agent. F) Experimental setup including a *S. gregaria* individual and a robotic cue, the virtual sections of the experimental arena, as well as the external robotic platform.

$P < 0.0001$) and INB ($Z = -8.67$; $P < 0.0001$). Locusts spent more time in the robot section with IB than with INB ($Z = -7.96$; $P < 0.0001$).

The time spent by *S. gregaria* individuals in the exploration section was significantly influenced by different artificial agents ($\chi^2 = 96.09$, $d.f. = 3$, $P < 0.0001$). Locusts spent significantly more time in the exploration section with HNB than with HB ($Z = 8.13$; $P < 0.0001$), IB ($Z = 6.83$; $P < 0.0001$), and INB ($Z = 5.73$; $P < 0.0001$). The time spent in the exploration section was significantly shorter with HB than with IB ($Z = -5.7$; $P < 0.0001$) and INB ($Z = -4.25$; $P = 0.0001$).

The time spent by *S. gregaria* individuals in the release section was importantly affected by different artificial agents ($\chi^2 = 163.64$, $d.f. = 3$, $P < 0.0001$). The period spent by locusts in the release section was longer with INB than with HB ($Z = 8.61$; $P < 0.0001$), HNB ($Z = 8.3$; $P < 0.0001$), and IB ($Z = 3.8$; $P = 0.0008$). The time spent in the release section was significantly longer with IB than with HB ($Z = 8.61$; $P < 0.0001$) and HNB ($Z = 8.48$; $P = 0.0001$). When HNB was exposed, the time spent by locusts in the release section was longer than when HB was exposed ($Z = 7.42$; $P = 0.0001$).

Different artificial agents significantly impacted grooming behavior in locusts ($\chi^2 = 143.94$, $d.f. = 3$, $P < 0.0001$). In particular, infected agents evoked a longer grooming behavior. Grooming behavior was performed longer with IB than with HB ($Z = 8.42$; $P = 0.0001$) and HNB ($Z = 8.43$; $P = 0.0001$). Also, grooming was longer with INB than with HB ($Z = 8.51$; $P = 0.0001$) and HNB ($Z = 8.47$; $P = 0.0001$) (Figure 2B).

The duration of tactile interaction with robotic cues was significantly different with different artificial agents ($\chi^2 = 63.67$, $d.f. = 3$, $P < 0.0001$). Tactile interaction was performed significantly longer toward IB than toward HB ($Z = 4.21$; $P < 0.0001$), HNB ($Z = 6.62$; $P < 0.0001$), and INB ($Z = 5.67$; $P < 0.0001$). Also, locusts performed longer tactile interaction toward HB than toward HNB ($Z = 3.79$; $P = 0.0009$) (Figure 2C).

Concerning aggressive interactions, we noted significant differences in the number of kicks given against different artificial agents ($\chi^2 = 14.23$, $d.f. = 3$, $P = 0.0026$). The number of kicks given against HB was lower than those given against INB ($Z = -3.58$; $P = 0.0019$), HNB ($Z = -2.83$; $P = 0.0238$), and IB ($Z = -2.78$; $P < 0.0271$) (Figure 2D).

Locusts post-exposed to IB spent more time in the release section than in the exploration section ($Z = 8.61$; $P < 0.0001$), in the robot section ($Z = 8.62$; $P < 0.0001$), and more time in the exploration section than in the robot section ($Z = 6.73$; $P < 0.0001$) (**Figure** 3A).

Locusts post-exposed to HB spent more time in the robot section than in the release section ($Z = 8.61$; $P < 0.0001$), in the exploration section ($Z = 8.61$; $P < 0.0001$), and more time in the release section than in the exploration section ($Z = 7.79$; $P < 0.0001$) (Figure 3B).

Locusts post-exposed to INB spent more time in the release section than in the exploration section ($Z = 8.52$; $P < 0.0001$), in the robot section ($Z = 8.67$; $P < 0.0001$), and more time in the exploration section than in the robot section ($Z = 8.18$; $P < 0.0001$) (Figure 3C).

Locusts post-exposed to HNB spent more time in the release section than in the exploration section ($Z = 3.8$; $P = 0.0004$), in the robot section ($Z = 6.19$; $P < 0.0001$), and more time in the exploration section than in the robot section ($Z = 3.6$; $P = 0.0009$) (Figure 3D).

2.2. Impact of Different Robotic Cues on Infected Locusts' Behavioral Response

Herein, we investigated the behavior of infected locusts post exposure to different artificial agents inoculated with the entomopathogenic fungus.

Different artificial agents did not significantly affected the latency duration in infected locusts ($\chi^2 = 0.53$, $d.f. = 3$, $P = 0.91$) (Figure 3A).

The time spent by infected locusts in the robot section was significantly affected by different artificial agents ($\chi^2 = 91.25$,

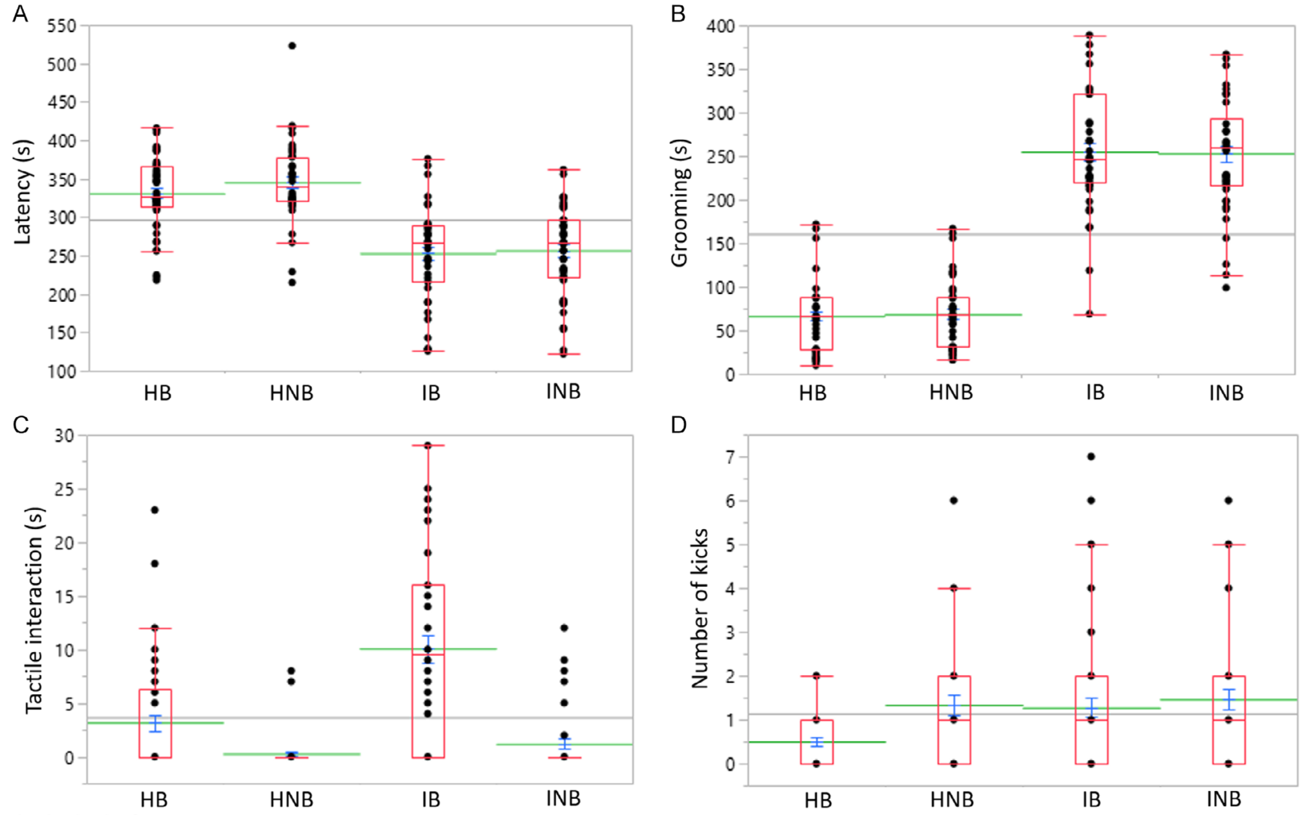

Figure 2. Responses of healthy *Schistocerca gregaria* individuals to different robotic cues. Effects that the infected biomimetic agent (IB), the healthy biomimetic agent (HB), the infected non-biomimetic agent (INB), and the healthy non-biomimetic agent (HNB) had on the duration of A) latency, B) grooming behavior, C) tactile interaction display, and D) on the kicking behavior in healthy locusts. In each box plot, the red line indicates the median and its dispersion range, encompassing the lower and upper quartiles, including outliers. The green line represents the mean, while the blue T bars indicate the standard error value.

d.f. = 3, $P < 0.0001$) that showed a preference for the biomimetic agents whether or not the pathogen is present. Infected locusts spent a longer time in the robot section with HB than with HNB ($Z = 7.61$; $P < 0.0001$), and INB ($Z = 5.88$; $P < 0.0001$). Infected locusts spent a longer time in the robot section with IB than with HNB ($Z = 6.96$; $P < 0.0001$), and INB ($Z = 4.92$; $P < 0.0001$), and longer with INB than with HNB ($Z = 4.4$; $P < 0.0001$).

The time spent by infected locusts in the exploration section was importantly affected by different artificial agents ($\chi^2 = 47.74$, *d.f.* = 3, $P < 0.0001$). Infected locusts spent a shorter time in the exploration section with HB than with HNB ($Z = -5.82$; $P < 0.0001$), and INB ($Z = 3.43$; $P = 0.0033$). Infected locusts spent a longer time in the exploration section with HNB than with IB ($Z = 5.51$; $P < 0.0001$), and INB ($Z = 2.67$; $P = 0.0375$). The time spent in the exploration section was shorter with IB than with INB ($Z = -3.4$; $P = 0.0037$).

The time spent by infected *S. gregaria* individuals in the release section was importantly influenced by different artificial agents ($\chi^2 = 23.24$, *d.f.* = 3, $P < 0.0001$). When HB was exposed, infected locusts spent a shorter time in the release section than when HNB ($Z = -5.09$; $P = 0.0002$), and INB ($Z = -3.25$; $P = 0.0062$) were present in the arena. The time spent by infected locusts in the release section was shorter with IB than with HNB ($Z = -3.45$; $P = 0.0031$).

Grooming behavior in infected *S. gregaria* individuals was not significantly impacted by different artificial agents ($\chi^2 = 1.27$, *d.f.* = 3, $P = 0.73$) (Figure 3B).

However, we found significant differences in the duration of tactile interaction with different artificial agents ($\chi^2 = 63.67$, *d.f.* = 3, $P < 0.0001$). Infected locusts performed tactile interaction longer toward IB than toward HB ($Z = 3.41$; $P = 0.0036$), HNB ($Z = 7.87$; $P < 0.0001$), and INB ($Z = 6.76$; $P < 0.0001$). Infected locusts performed tactile interaction longer toward HB than toward HNB ($Z = 6.37$; $P = 0.0036$), INB ($Z = 4.63$; $P < 0.0001$) (**Figure** 4C).

Different artificial agents did not significantly affected the number of kicks given by infected locusts during aggressive interactions ($\chi^2 = 1.72$, *d.f.* = 3, $P = 0.63$) (Figure 4D).

Infected locusts post-exposed to IB spent more time in the robot section than in the exploration section ($Z = 8.01$; $P < 0.0001$) and in the release section ($Z = 7.7$; $P < 0.0001$) (**Figure** 5A).

Infected locusts post-exposed to HB spent more time in the robot section than in the exploration section ($Z = 8.39$;

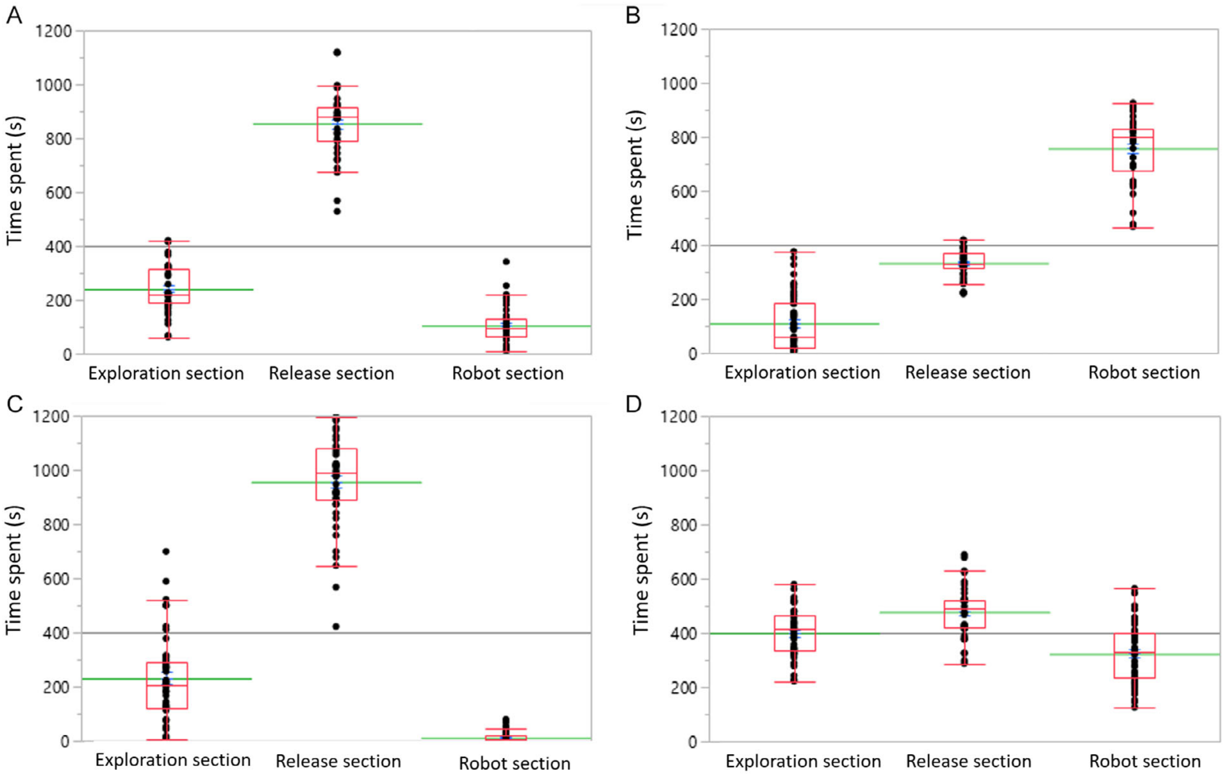

Figure 3. Placing duration of healthy *Schistocerca gregaria* individuals across different sections of the experimental arena affected by different robotic cues. Time spent by healthy locusts in the robot section, the exploration section, and release section of the experimental arena post-exposed to A) the infected biomimetic agent (IB), B) the healthy biomimetic agent (HB), C) the infected non-biomimetic agent (INB), and D) the healthy non-biomimetic agent (HNB). In each box plot, the red line indicates the median and its dispersion range, encompassing the lower and upper quartiles, including outliers. The green line represents the mean, while the blue T bars indicate the standard error value.

$P < 0.0001$) and in the release section ($Z = 8.25$; $P < 0.0001$) (Figure 5B).

Infected locusts post-exposed to INB spent more time in the robot section than in the exploration section ($Z = 5.84$; $P < 0.0001$) and in the release section($Z = 5.55$; $P < 0.0001$) (Figure 5C).

The time spent in different section by infected locusts post-exposed to HNB was not significantly different ($\chi^2 = 1.4$, $d.f. = 2$, $P = 0.49$) (Figure 5D).

2.3. Differences between Healthy and Infected Locusts' Response to Robotic Cues

Behavioral responses of healthy and infected *S. gregaria* individuals were often significantly diverse during the interaction with different artificial agents (**Table 1**).

During the interaction with HB, the duration of latency was longer in healthy locusts (331.24 s) than in infected locusts (179.4 s). Also, the time spent in the robot section was longer in healthy locusts (757.84 s) than in infected locusts (570.54 s), while the time spent in the exploration section was shorter in healthy locusts (108.84 s) than in infected locusts (308.68 s). We did not observe significant differences in the time spent by healthy (333.32 s) and infected locusts (320.78 s) in the release section. Infected locusts performed grooming behavior for a longer period (524.72 s) than healthy locusts (66.48 s), as well as tactile interactions lasted more in infected locusts (6.34 s) than in healthy locusts (3.16 s). Infected locusts resulted also more aggressive against HB, performing an higher number of kicks (1.82) than healthy locusts (0.5).

When IB was presented, the latency duration was longer in healthy locusts (253.26 s) than in infected locusts (172.9 s). Infected locusts spent more time in the robot section (565.7 s) than healthy locusts (104.24 s), as well as in the exploration section (i.e., infected locusts: 299.16 s; healthy locusts: 240.94 s), while healthy locusts spent more time in the release section (854.82 s) than infected locusts (335.14 s). Grooming behavior lasted significantly longer in infected locusts (528.9 s) compared to healthy individuals (255.2 s), but not significant differences between infected and healthy locusts where noted in the duration of tactile interactions (i.e., healthy locusts: 10.08 s; infected locusts: 14.16 s). A higher number of kick acts were recorded in infected locusts (1.84) compared to healthy locusts (1.28).

When locusts interacted with HNB, latency duration was longer in healthy individuals (345.38 s) than in infected ones (176.76 s). The time spent in the robot section was longer in infected locusts (376.56 s) than in healthy ones (322.92 s), and that in the release section was longer in healthy locusts (477.77 s) than in infected locusts (414.2 s), while no significant differences were noted in the time spent by healthy (399.38 s)

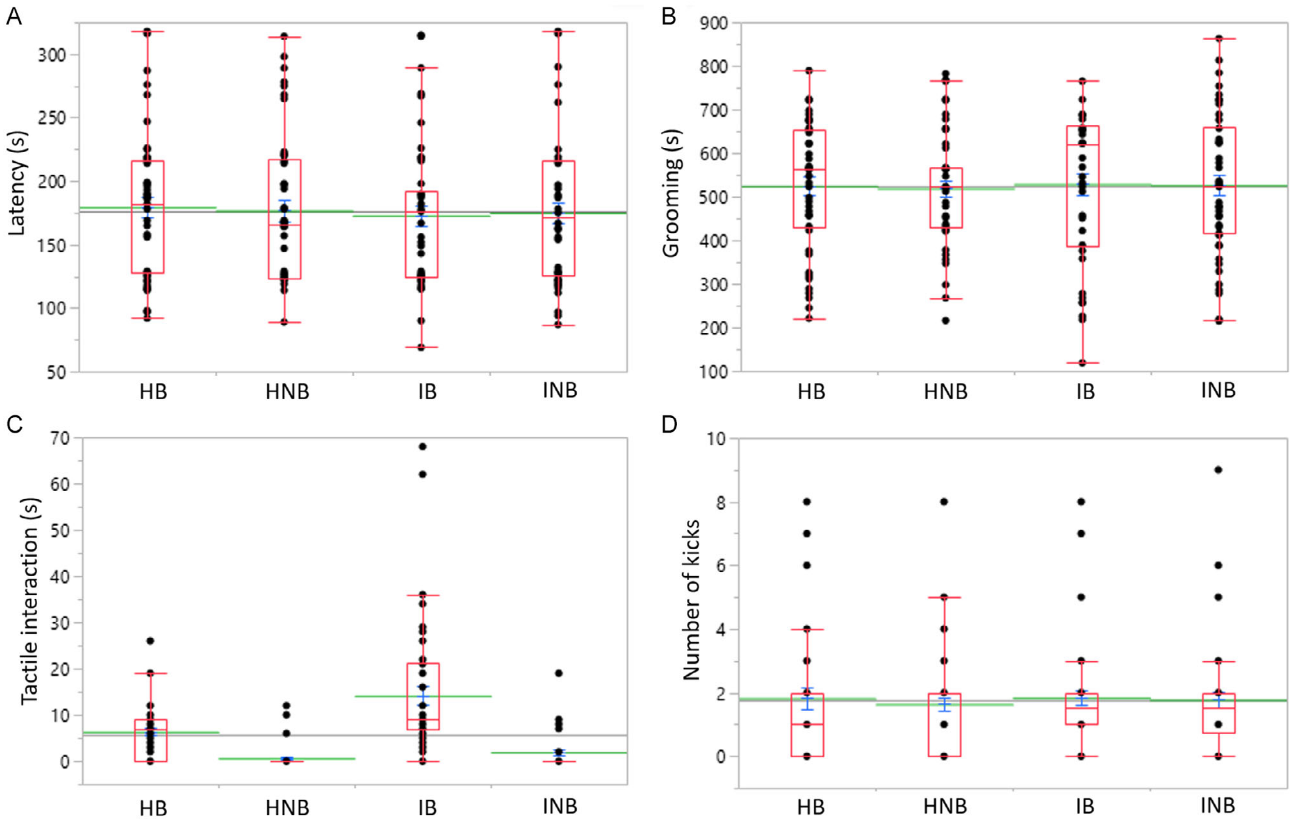

Figure 4. Responses of infected *Schistocerca gregaria* individuals to different robotic cues. Effects that the infected biomimetic agent (IB), the healthy biomimetic agent (HB), the infected non-biomimetic agent (INB), and the healthy non-biomimetic agent (HNB) had on the duration of A) latency, B) grooming behavior, C) tactile interaction display, and D) on the kicking behavior in infected locusts. In each box plot, the red line indicates the median and its dispersion range, encompassing the lower and upper quartiles, including outliers. The green line represents the mean, while the blue T bars indicate the standard error value.

and infected locusts (409.24 s) in the exploration section. Infected locusts performed grooming behavior longer (518.86 s) than healthy locusts (69.06 s). No important differences were observed in the duration of tactile interaction (i.e., healthy locusts: 0.3 s; infected locusts: 0.53 s) and number of kicks (i.e., healthy locusts: 1.34; infected locusts: 1.64).

Exposures to INB elicited a longer latency duration in healthy locusts (331.24 s) than in infected ones (179.4 s). Healthy locust spend a shorter time in the robot section (12.21 s) compared to infected locusts (179.4 s). Furthermore, healthy locust spend a shorter time in the exploration section (230.96 s) compared to infected ones (363.24 s), while healthy locust stayed longer in the release section (956.76 s) than in the infected locusts (370.68 s). Grooming behavior was performed shorter in healthy locusts (252.92 s) than in infected ones (526.58 s), and no significant differences were recorded in the case of tactile interactions (i.e., healthy locusts: 1.22 s; infected locusts: 1.09 s), and number of kicks (i.e., healthy locusts: 1.46; infected locusts: 1.78).

3. Discussion

Our study proposes a bionic approach, employing the animal–robot interaction emerging paradigm,[45,46] to investigate the complex dynamics of social immunity in insects species forming large swarms, focusing on the gregarious phase of *S. gregaria*. Locusts are naturally attracted to artificial models of conspecifics.[47,48] In addition, recent studies have revealed locusts capacity for social learning when exposed to robotic agents designed to mimic conspecific appearance and behaviors.[46] Herein, the use of biomimetic agents inoculated with the entomopathogenic fungus *B. bassiana* allowed us to explore the behavioral responses of healthy locusts and those already infected, shedding light on potential group-level strategies to limit pathogen spread.

The behavioral responses observed in healthy locusts exposed to different artificial agents unveil new aspects of their interaction dynamics. Notably, the latency duration varied significantly, with healthy individuals exhibiting prolonged periods of immobility when exposed to both healthy and infected biomimetic agents. This finding suggests a cautious approach and heightened alertness in the presence of novel stimuli, highlighting the role of individual surveillance behaviors in the swarm as part of the host's immune defenses,[49] as well as the risk of cannibalism in swarming locusts.[50] Interestingly, while latency increased, locusts showed a clear preference for spatial occupation, spending more time in the robot section when exposed to healthy biomimetic agents. This spatial affinity suggests that,

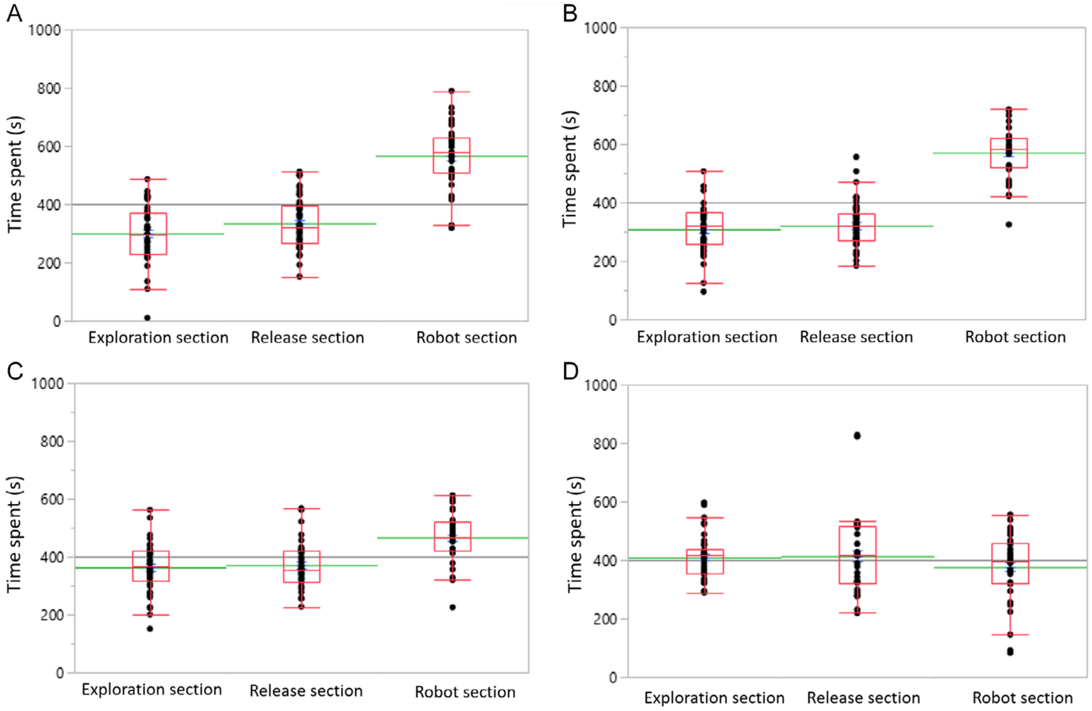

Figure 5. Placing duration of infected *Schistocerca gregaria* individuals across different sections of the experimental arena affected by different robotic cues. Time spent by infected locusts in the robot section, the exploration section, and release section of the experimental arena post-exposed to A) the infected biomimetic agent (IB), B) the healthy biomimetic agent (HB), C) the infected non-biomimetic agent (INB), and D) the healthy non-biomimetic agent (HNB). In each box plot, the red line indicates the median and its dispersion range, encompassing the lower and upper quartiles, including outliers. The green line represents the mean, while the blue T bars indicate the standard error value.

despite the increased caution, locusts still recognize the biomimetic agents as conspecific-like entities. This behavior is consistent with previous research showing that locusts exhibit an innate attraction to conspecific-like objects, even when they are not fully familiar or "real" conspecifics.[46–48] Such behavior highlights the complex nature of locust responses based on both familiarity and perceived threat. These findings also reinforce the potential of biomimetic agents as useful tools in studying swarm dynamics and group behaviors in locusts.

Additionally, the time spent grooming and engaging in tactile interactions increased significantly in the presence of infected biomimetic agents, indicating heightened social interactions among healthy individuals as a potential preventive measure against pathogen transmission. Tactile interaction behavior, a crucial aspect of social immunity,[51] was notably extended toward infected agents. This heightened grooming may represent an adaptive response to the entomopathogenic fungus, emphasizing the importance of reciprocal hygiene in limiting the spread of pathogens within the swarm.

The observed aggressive acts, as indicated by the number of kicks, were notably lower when healthy locusts interacted with healthy biomimetic agents compared to other artificial agents. This interesting result hints at a potential segregation mechanism within the swarm.

Infected locusts have been reported to display altered behavioral responses in different contexts. Previous research reported effects on intrasexual interactions in infected locusts such as an increased male–male mounting behavior.[52] This phenomenon can be due to a last reproduction attempt of sick males, as well as to their lowered attractiveness to females.

Herein, latency duration remained consistent across agents, suggesting that the infection status did not significantly influence the initial reaction time. Interestingly, infected locusts exhibited a preference for the robot and exploration sections, potentially indicating a response to the stimuli generated by the robotic agents, biomimetic or not. Infected locusts tended to interact more with robotic conspecifics (both infected and healthy), perhaps to increase the likelihood of having the fungal conidia removed. However, the extended time spent in these sections highlights the potential for infected individuals to engage with their environment, and this behavior could expose them more easily to predators, reducing the number of infected individuals in the swarm.

One consideration in the current study is that we did not measure baseline behaviors between two healthy locusts as a direct comparison. While previous work from our group has demonstrated that our biomimetic agents effectively influence locust behavior, including interactions related to social learning and predator avoidance,[40] baseline data on healthy locusts interacting with each other would provide a useful reference for further validating the biohybrid approach.

Our findings align with broader evidence of infection-induced alterations in social behaviors across group-living species. Similar to locusts' responses to pathogen-inoculated biomimetic

Table 1. Healthy versus infected locusts' behavioral responses post-exposure to different robotic cues (Wilcoxon test; $P < 0.05$). IB = infected biomimetic agent; HB = healthy biomimetic agent; INB = infected non-biomimetic agent; and HNB = healthy non-biomimetic. Red boxes indicate nonsignificant values.

Robotic cue		Latency [s]	Robot section [s]	Exploration section [s]	Release section [s]	Grooming [s]	Tactile interaction [s]	Kicks [*n*]
HB	Healthy locusts	331.24 ± 6.81	757.84 ± 16.49	108.84 ± 14.59	333.32 ± 6.81	66.48 ± 5.43	3.16 ± 0.74	0.5 ± 0.09
	Infected locusts	179.4 ± 7.87	570.54 ± 11.53	308.68 ± 10.89	320.78 ± 10.76	524.72 ± 20.76	6.34 ± 0.79	1.82 ± 0.32
	$\chi 2$	66.637	47.537	53.205	2.166	74.314	11.797	12.905
	d.f.	1	1	1	1	1	1	1
	P	<0.0001	<0.0001	<0.0001	0.141	<0.0001	0.0006	0.0003
IB	Healthy locusts	253.26 ± 8.16	104.24 ± 9.17	240.94 ± 12.79	854.82 ± 17.49	255.2 ± 9.45	10.08 ± 1.27	1.28 ± 0.22
	Infected locusts	172.9 ± 7.82	565.7 ± 15.06	299.16 ± 13.08	335.14 ± 12.23	528.9 ± 24.71	14.16 ± 1.97	1.84 ± 0.24
	$\chi 2$	34.93	73.916	11.438	74.275	46.038	1.209	4.315
	d.f.	1	1	1	1	1	1	1
	P	<0.0001	<0.0001	0.0007	<0.0001	<0.0001	0.271	0.037
HNB	Healthy locusts	345.38 ± 6.92	322.92 ± 15.07	399.38 ± 12.77	477.7 ± 12.87	69.06 ± 5.43	0.3 ± 0.21	1.34 ± 0.23
	Infected locusts	176.76 ± 8.45	376.56 ± 15.51	409.24 ± 10.46	414.2 ± 17.86	518.86 ± 18.02	0.56 ± 0.32	1.64 ± 0.21
	$\chi 2$	70.298	7.211	0.07	9.123	74.291	0.225	3.479
	d.f.	1	1	1	1	1	1	1
	P	<0.0001	0.0072	0.79	0.0025	<0.0001	0.635	0.062
INB	Healthy locusts	331.24 ± 6.81	12.28 ± 2.57	230.96 ± 22.26	956.76 ± 23.63	252.92 ± 9.01	1.22 ± 0.43	1.46 ± 0.22
	Infected locusts	179.4 ± 7.86	466.08 ± 10.55	363.24 ± 11.68	370.68 ± 10.89	526.58 ± 22.67	1.09 ± 0.57	1.78 ± 0.26
	$\chi 2$	33.867	75.305	26.415	72.857	58.832	0.601	1.567
	d.f.	1	1	1	1	1	1	1
	P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.4379	0.2106

agents, research on fish has shown that parasite infections can disrupt collective dynamics. Infected individuals in fish shoals impair escape wave transmission, increasing predation risks for their groups,[18] and elicit avoidance behaviors that highlight the role of social distancing in mitigating disease spread.[19] Additionally, parasitic infections alter movement patterns, influencing group cohesion and leadership dynamics.[20] These parallels underscore the widespread nature of infection-driven social adaptations across taxa and reinforce the utility of biohybrid approaches to probe the mechanisms underlying social immunity in collective systems.

Our study introduces the innovative concept of using robotic agents to examine social immunity in locust swarms, providing a controllable and standardized experimental platform. The bionic paradigm presented here opens avenues for further investigations into the complex interplay between individual and group-level behaviors in the context of infectious diseases within social and gregarious insect population.[53] Our findings underscore the importance of individual and social behaviors in shaping the social immunity of insect group complex systems. Science-based robotics, combining technology and ethology, offers a powerful tool to unravel the complexity of social interactions and disease responses in group-living organisms, further establishing robotics as a transformative approach for groundbreaking scientific research.[54] Future studies may build upon these insights to develop targeted strategies for managing and mitigating infectious diseases in social insect populations, with potential applications in agriculture and pest control. In addition, this research can be applied to enhance computational methods that model the dynamics of diseases, leading to a more precise evaluation of the spread of both established and newly emerging pathogens in both human and wildlife environments.[55,56]

4. Summary

This study investigates locusts' interactive behavioral adaptations as a strategy to limit pathogen spread within swarms, providing novel insights into the dynamics of social immunity. By employing an innovative animal–robot interaction paradigm, we investigated locusts responses to infected biomimetic agents, showcasing the potential for collective defense strategies in non-eusocial organisms. This research advances our understanding of social immunity behaviors and demonstrates the interdisciplinary potential of biomimetic robotics for studying complex behavioral phenomena, with implications for evolutionary ecology and pest management.

5. Experimental Section

Locust Rearing, Pathogen, and General Observations: Locusts were maintained at the BioRobotics Institute of Scuola Superiore Sant'Anna in controlled conditions (25 ± 1 °C, 55 ± 5% relative humidity, and with a 16:8 h L:D photoperiod) and fed *ad libitum* with a diet consisting in wheat, vegetables, and water. Adult *S. gregaria* of both sexes were used in the experiments. The experimental phase presented the same laboratory conditions reported earlier. The intensity of light was about 1000 lux around the testing arena. Locust–robot interactions were focally recorded by an observer.[57]

The entomopathogenic fungus used in the tests consisted in the *B. bassiana* (ATCC strain 74 040, containing 2.3×10^7 conidia mL^{-1}, originating from United States) from the commercial bioinsecticide Naturalis [CBC (Europe) S.r.l., Italy]. *B. bassiana* is a biological control agent of locusts.[25]

Biomimetic Agent and Robotic Platform: Visual cues have an important role in locusts gregarization.[22] So, morphometric traits of the model organism were used during the design of the biomimetic agent that reproduced the head, thorax, abdomen, two forelegs, two middle legs, two hindlegs, two tegmina, and two antenna of adults *S. gregaria*. The biomimetic agent prototype was designed using a solid modeling computer-aided design (e.g., SolidWorks, Dassault Systemes, Vélizy Villacoublay, France) and fabricated in a biocompatible resin (VisiJet M3 Crystal, 3D Systems) by additive manufacturing. The length of the biomimetic agent was 57 mm (e.g., from the distal end of the head to the distal end of tegmina). The same fabrication process was used to develop the non-biomimetic agent (57 mm long), consisting in a white object with an elliptical silhouette.[46]

The visual resemblance of the integumental color pattern of gregarious *S. gregaria* adults was obtained by painting the biomimetic agent with nontoxic pigments (Figure 1A–C).

The biomimetic agent was magnetically coupled with an external robotic platform located below the testing arena floor that generated the trajectory. The operating area of the robotic platform was around 400 × 200 mm, plotting the path with high accuracy (e.g., 0.01 mm). It was composed of two axes, each of them actuated by a stepper motor. The activation and control of the robotic platform were carried out by a microcontroller (Arduino Nano) that received the plotted trajectories converted in G-code (i.e., RS-274). The plotting and code conversion phases were addressed by an external processor connected to the microcontroller.

Insect–Robot–Pathogen Hybrid Interaction Experiments: Noninvasive topical inoculations of 2 µl of the commercial *B. bassiana* bioinsecticide were released on the dorsal cuticle of insects to test infected locusts.[28] An equal amount of sterile distilled water was inoculated on insects used to test healthy locusts.

Robotic cues exposed to locusts included an infected biomimetic agent (IB) and a healthy (untreated) biomimetic agent (HB) as a control. Furthermore, we exposed an infected non-biomimetic agent (INB) and a healthy non-biomimetic agent (HNB).

Infected agents were obtained by inoculating with a micro-applicator 2 µL of the commercial *B. bassiana* bioinsecticide on a small rectangle of filter paper dorsally embedded on the agents (Figure 1 D). Control healthy agents were inoculated with an equal amount of sterile distilled water.

The floor of the experimental arena (500 × 500 mm) was virtually divided in three equal sections (e.g., robot section, exploration section, release section). Individuals of *S. gregaria* were gently placed in the center of the release section and caged in an opaque cubic box (100 × 100 × 100 mm) for 5 min of acclimatation before the experiment. The biomimetic agent was placed in the robot section to magnetic couple with the robotic platform below (Figure 1E,F). When the experiment started, the opaque cubic box was lifted, and the biomimetic agent was activated, following an elliptical path (semi-major axis 175 mm; semi-minor axis 75 mm), with a velocity of 5 mm s^{-1} for 60 s interspersed with 30 s pauses in which it remained stationary.

During the biohybrid interaction, the following locust's behaviors were recorded: duration of latency (e.g., time needed to produce any motor display once the experiment started); time spent by *S. gregaria* individuals in the three sections of the experimental arena; duration of grooming (e.g., individuals engaged in cleaning their own body parts); duration of tactile interaction with the agents (e.g., individuals keeping their body in contact with the agents, and/or engaged in cleaning the agents' body parts); and number of aggressive acts toward the agents (e.g., kicks with hindlegs).

The duration of each test was 20 min. For each robotic cue, 50 healthy locusts and 50 infected locusts were analyzed. Each insect was tested only once.

Data Analysis: We used nonparametric statistics to analyze data collected on the impact of different robotic cues on *S. gregaria* individuals' behavior (e.g., latency duration, time spent in the three different sections, duration of grooming, duration of tactile interaction with the agents, as well as the number of aggressive acts), as they were distributed nonparametrically (Shapiro–Wilk test, goodness of fit $P < 0.05$). Both for healthy and for infected locusts, data on their behavioral responses to robotic cues, were analyzed via Wilcoxon/Kruskal–Wallis test followed by Steel–Dwass test ($P < 0.05$). To analyze behavioral differences between healthy and infected locusts, we relied on Wilcoxon test ($P < 0.05$). R software v4.2.0 supported data analysis.

Ethics Statement: This study adheres to the Guidelines for the Use of Animals in Research.[58] It also complies with the legal requirements outlined in Italian legislation (D.M. 116/1992) and relevant EU regulations.[59] No specific authorizations in using locusts were required in the country where the study was conducted.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Donato Romano: conceptualization (lead); data curation (equal); formal analysis (lead); funding acquisition (lead); investigation (lead); methodology (lead); project administration (lead); supervision (lead); writing—original draft (lead); and writing—review and editing (equal). **Cesare Stefanini**: data curation (equal); formal analysis (supporting); investigation (supporting); methodology (supporting); and writing—review and editing (equal).

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

animal–robot interaction, biohybrid system, insects, locusts, social immunity

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