environmental microbiology

Environmental Microbiology (2022) **24**(1), 507–516 doi:10.1111/1462-2920.15883

Microbiome-related aspects of locust density-dependent phase transition

Omer Lavy,[1†] **Ohad Lewin-Epstein**,[2†] **Yonatan Bendett**,[2] **Uri Gophna**,[3] **Eran Gefen**,[4] **Lilach Hadany**[2*] **and Amir Ayali**[1*]

[1]*School of Zoology, Tel Aviv University, Tel Aviv, Israel.*

[2]*Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv, Israel.*

[3]*Shmunis School of Biomedicine and Cancer Research, Tel Aviv University, Tel Aviv, Israel.*

[4]*Department of Biology, University of Haifa – Oranim, Kiryat Tivon, Israel.*

Summary

Locust plagues are a notorious, ancient phenomenon. These swarming pests tend to aggregate and perform long migrations, decimating cultivated fields along their path. When population density is low, however, the locusts will express a cryptic, solitary, non-aggregating phenotype that is not considered a pest. Although the transition from the solitary to the gregarious phase has been well studied, associated shifts in the locust's microbiome have yet to be addressed. Here, using 16S rRNA amplicon sequencing, we compared the bacterial composition of solitary desert locusts before and after a phase transition. Our findings revealed that the microbiome is altered during the phase transition, and that a major aspect of this change is the acquisition of *Weissella* (Firmicutes). Our findings led us to hypothesize that the locust microbiome plays a role in inducing aggregation behaviour, contributing to the formation and maintenance of a swarm. Employing a mathematical model, we demonstrate the potential evolutionary advantage of inducing aggregation under different conditions; specifically, when the aggregation-inducing microbe exhibits a relatively high horizontal transmission rate. This is the first report of a previously unknown and important aspect of locust phase transition, demonstrating that the phase shift includes a shift in the gut and integument bacterial composition.

Received 21 February, 2021; revised 16 November, 2021; accepted 21 December, 2021. *For correspondence. E-mail lilach.hadany@gmail.com; Tel. +972-3-640-9831; Fax +972-36406886. E-mail ayali@tauex.tau.ac.il; Tel. +972-3640576; Fax +972-36409403. †These authors contributed equally to this work.

Introduction

Locusts are best known for their devastating potential to cause immense damage to both natural and cultivated vegetation. These short-horned grasshoppers (Orthoptera), belonging to the family Acrididae, frequently aggregate and migrate in swarms, decimating crops over large areas of the developing world (Ayali, 2019; Le Gall *et al*., 2019; Zhang *et al*., 2019, FAO Locust Watch: http://www.fao.org/ag/locusts). All locust species may express two very different density-dependent phenotypes (or phases): at low densities, locusts behave in a cryptic manner, actively avoiding interaction with conspecifics and generally not posing a serious threat to crops (i.e. the solitary phase). When crowded, however, locusts express the notorious gregarious phenotype, tending to aggregate into swarms, to migrate, and to consume huge quantities of vegetation, resulting in severe damage to agriculture (the gregarious phase) (Pener and Simpson, 2009; Cullen *et al*., 2017; Le Gall *et al*., 2019; Zhang *et al*., 2019). The phase transition from solitary to gregarious in the different locust species encompasses many changes, including a marked behavioural change (e.g. Pener and Simpson, 2009; Cullen *et al*., 2017; Golov *et al*., 2018; Knebel *et al*., 2019), as well as immense physiological changes (e.g. Ayali and Pener, 1995; Pener and Simpson, 2009; Talal *et al*., 2015; Cullen *et al*., 2017).

Among the studies investigating the different characteristics of the locust density-dependent phase phenomenon, very few have addressed the microbiome-related aspects. The role of gut bacteria in maintaining swarm integrity, through the emission of locust-attracting faecal volatiles, was suggested by Dillon *et al*. (2000, 2002). Recently, Lavy *et al*. (2019, 2020a) reported the occurrence of phase-related different temporal dynamics in bacterial composition of the gut and the reproductive tract in the desert locust (*Schistocerca gregaria*). No study to date, however, has directly addressed the bacterial-composition-related aspects of the locust density-dependent phase transition.

Recent studies have revealed various mechanisms by which the microbiome can affect host behaviour (Dinan and Cryan, 2017; Kraimi *et al*., 2019), including in insects (Yuval, 2017; Liberti and Engel, 2020). Other studies have explored the evolutionary perspective of microbiome-induced social interactions (Lewin-Epstein *et al*., 2017; Gurevich *et al*., 2020; Lewin-epstein and Hadany, 2020). In the current controlled laboratory study, we used the desert locust (*Schistocerca gregaria*) as a model with which we directly explored the association between solitary-to-gregarious phase transition and the individual locust's microbiome. We then devised a mathematical model with which we examined the conditions that may favour the evolution of microbial species that induce aggregation behaviour in their host. We found that microbes with an advantage in horizontal transmission would benefit from inducing increased interaction rates and aggregation in their hosts, thus improving their transmission potential. This in turn will result in locust crowding and, in parallel with changes in population density, could facilitate the locust swarming behaviour.

Results

Gregarious and solitary locust bacterial composition analysis

Our first step towards exploring the bacterial aspects of locust phase shift was sequencing the integument and gut bacterial composition of 21 solitary locusts, before ($t = 0$ days) and after crowding them with gregarious conspecifics for 7 days ($t = 7$ days). We then compared these to the integument and gut bacterial composition of 21 crowd-reared gregarious locusts (see Experimental procedures).

We found that the microbiome of both the gregarious and the solitary locust faeces samples was comprised primarily of members of the Proteobacteria and Firmicutes (Fig. 1A). The locust integument also featured a representation of Actinobacteria and Bacteroidetes (Fig. 1B). We further observed that while the bacterial composition of the solitary locusts was very different from that of the gregarious control group at $t = 0$, these differences had diminished by $t = 7$. This striking shift in the solitary locust's gut and integument bacterial composition could be mostly attributed to an increase in Firmicutes during the 7 days of gregarious crowding (Fig. 1).

This trend in bacterial shift of solitary-to-gregarious locusts was also observed at the genus level: the integument bacterial composition of the solitary locusts notably differed from that of the gregarious locusts at $t = 0$ (ANOSIM: $p < 0.001$, $R = 0.43$; Fig. 2A). By the end of the crowding period, however, the locusts of solitary origin had lost their unique bacterial composition and

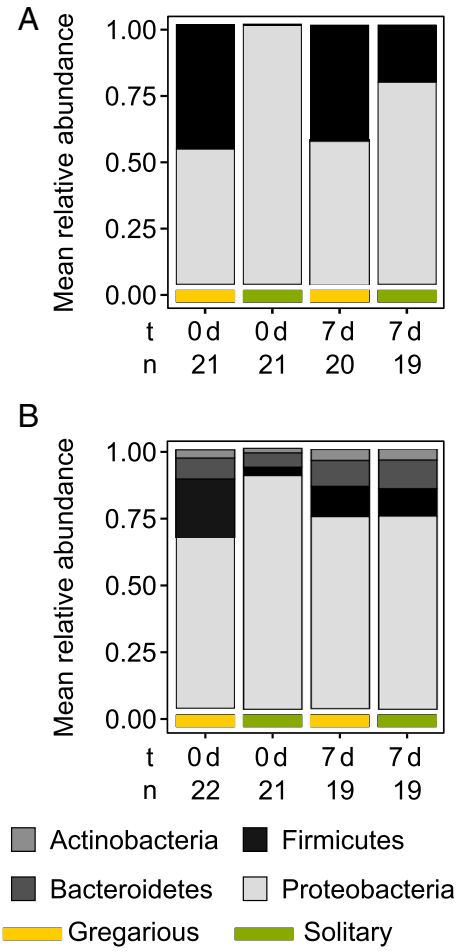

Fig 1. Mean relative abundance of the dominant phyla comprising the bacterial composition of the faeces (A) and the integument (B) of gregarious-grown (yellow underscore) and solitary-grown (green underscore) individuals at $t = 0$ d and at $t = 7$ d, where $t = 0$ d is the time when the solitary-grown locusts were moved to crowding conditions. It is noticeable that the phylum Firmicutes is absent from the faeces of the solitary locusts at $t = 0$ and present after 7 days. In addition, while the faeces bacteria of both gregarious and solitary locusts feature predominantly Firmicutes and Proteobacteria, the locust's integument is also populated with bacteria from the Actinobacteria and Bacteroidetes phyla.

displayed a gregarious-like bacterial profile on their integument (ANOSIM: $p = 0.34$, $R = 0.005$; Fig. 2A1).

A constrained canonical analysis and analysis of similarities of the locust's gut bacterial composition revealed the same pattern of transition from a distinct solitary gut microbiota at $t = 0$ (ANOSIM: $p < 0.001$, $R = 0.51$; Fig. 2B) to bacterial compositions that were much closer to those of gregarious locusts after 7 days of crowding (ANOSIM: $p = 0.037$, $R = 0.08$; Fig. 2B1). Most notably, the genus *Weissella* (Firmicutes) was absent from the gut of solitary locusts at $t = 0$, whereas it dominated in the gregarious population, contributing considerably to the observed per-phase difference before crowding (Fig. 2B and C). In contrast, at $t = 7$ the contribution of *Weissella*

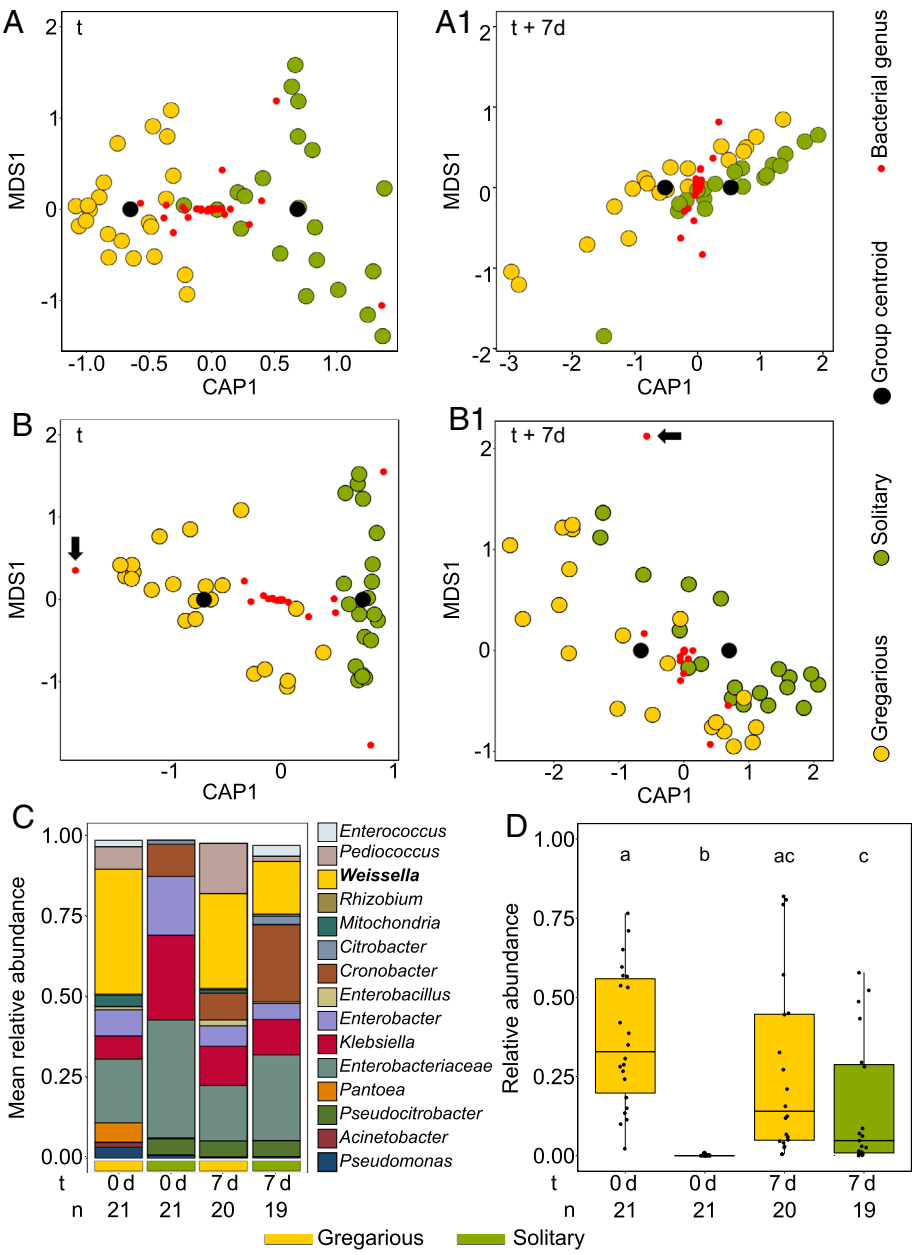

Fig 2. Genus level, Bray–Curtis based, Constrained Canonical Analysis of integument bacterial composition according to phase at $t = 0$ d (A) and $t = 7$ d (A1), and of the faeces bacterial composition at $t = 0$ d (B) and $t = 7$ d (B1). The *x*-axis displays the variation of the explanatory variables (i.e. the phase groups). The location of the bacterial genera (red dots) relative to the group centroids (black) represents the between-group variability explained by a specific bacterial genus. At $t = 0$ d, the genus *Weissella* (marked with a black arrow) is a main contributor to the between-phase variance (based on its location on the *x*-axis) in the faeces bacterial composition (B); whereas, at $t + 7$ d it is located almost between the group centroids, decreasing dramatically in its contribution to the variance (B1).
C. Mean relative abundance of the dominant genera comprising the faeces bacterial composition of gregarious (yellow underscore) and solitary (green underscore) at $t = 0$ d and $t = 7$ d. It is noticeable that the genus *Weissella* is absent from the solitary treatment at $t = 0$ but present after 7 days.
D. Relative abundance of the dominant ASV assigned to the genus *Weissella* in the gregarious and solitary-originated locusts. The *Weissella*-assigned ASV was absent from the solitary treatment at $t = 0$ days, whereas at $t = 7$ days there was no significant difference between the two treatments. Differences between the groups were analysed using the Kruskal–Wallis test ($p < 0.001$) and Dunn's test as post hoc.

to the per-phase difference strongly decreased, as it became prevalent also among the previously solitary locusts (Fig. 2B and C).

Higher-resolution analysis of the data revealed that a specific gregarious-prevalent amplicon sequence variant (ASV) had been acquired by the solitary locusts during the crowding period (Fig. 2D), confirming that this is indeed the same bacterium that was transmitted from the gregarious locusts to their solitary conspecifics during the gregarization process of the latter. This ASV was perfectly aligned to the parallel sequence segment of *Weissella cibaria* (top hit strain: KACC 11862, similarity: 100% across 429 bp).

Quantitative polymerase chain reaction (QPCR) of the absolute concentration of bacterial DNA in the samples did not show substantial differences in gut bacterial load between days 0 and 7 in either treatment (gregarious or solitary) (Fig. S1). This result indicates that the *Weissella* cells found in the solitary gut at day 7 have probably replaced other gut bacteria that were present at day 0, and suggests that the solitary gut microbiome became more gregarious-like in composition.

Both solitary and gregarious locusts showed increased integument bacterial amounts at day 7 in comparison to day 0. Indicating a process of bacterial accumulation, common for both treatments (Fig. S1).

Microbiome-induced aggregation model

In light of our findings, as well as findings from previous studies, revealing the presence of *Weissella* in gregarious locusts but not in solitary individuals, we raise the hypothesis that microbes may have evolved to induce aggregative behaviour of their hosts. Specifically, we suggest that microbes with relative advantage in horizontal transmission may benefit from eliciting and maintaining host crowding, which further facilitates their spread in the population. We, therefore, continued by devising a mathematical model in order to examine the potential evolutionary advantage of aggregation-inducing microbes under various environments, and the conditions that favour this trait.

We considered a fully mixed population of locusts, each carrying one of three microbiome compositions: a standard solitary microbiome composition (denoted by *S*); the standard microbiome plus microbes of type α that induce their hosts to aggregate (denoted by α) – to initiate more interactions than other hosts by a factor of $g>1$; and the standard microbiome plus microbes of type β which do not affect their host's behaviour (denoted by β). Locusts could lose microbes α and β at rates L_α, L_β respectively. We used a compartmental model, as described in the Experimental procedures section.

We began by analyzing the model described by Eqs. E1–E3 (see Experimental procedures), constructed to examine the possibility of bacterial effects on the behaviour of the locusts. We first considered populations with constant density ($D(t)=1$ throughout) and without mutations ($\mu=0$). In this case, we found analytical expressions for the equilibrium points of the system and their stability (see details in the Supplementary Information). The analysis revealed that in a competition between *S* and β alone, β can evolve and be maintained in polymorphism at a frequency of $1-\frac{L_\beta}{T_\beta}$ (the frequency of *S* would be $\frac{L_\beta}{T_\beta}$). We thus see that when $T_\beta \le L_\beta$, β becomes extinct. Similarly, with α and *S*, α can evolve and be maintained in polymorphism at a frequency of $1-\frac{L_\alpha}{T_\alpha \cdot g}$. In the case of α, it is not the horizontal transmission probability (T_α) alone that determines its success, but rather the effective transmission rate $(T_\alpha \cdot g)$, combining both the horizontal transmission probability and the induction of interactions rate. When all three types are competing in the population, we find that α can be maintained in polymorphism with *S* if the following two conditions are satisfied:

$$1.\ T_\alpha \cdot g > L_\alpha$$

$$2.\ T_\alpha \cdot g > T_\beta \cdot \left(g - \frac{L_\alpha}{T_\alpha}\left(1-\frac{1}{g}\right)\right) + L_\alpha - L_\beta$$

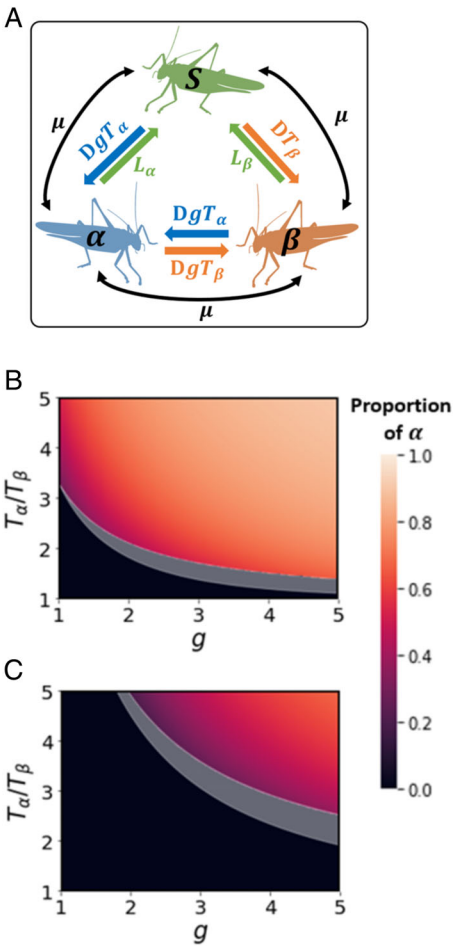

Fig 3. Model illustration and analytic results.
A. Illustration of the transition rates between the three microbiome types. T_α and T_β are the horizontal transmission rates of microbes α and β. g is the fold-increase in locust interactions due to the induction of aggregation by microbe α. L_α and L_β are the rates at which the locusts lose microbes α,β respectively. $D(t)$ denotes population density, affecting the rate of *all* interactions, while μ represents mutations in all possible directions.
B–C. The expected proportion of hosts bearing microbe α in the population, based on analysis of the equilibria of Equations (E1–E3) and their stability (see Supplementary Information) is plotted for $L_\alpha=0.06$ (B) and $L_\alpha=0.2$ (C). The black area represents the range of parameters in which α cannot evolve; the grey area represents the range of parameters in which α will either become extinct or reach polymorphic equilibrium, depending on the initial conditions of the population; and the coloured area represents the range of parameters in which α will evolve and reach stable polymorphism with *S*, regardless of the initial conditions of the population. $T_\beta=0.025, L_\beta=0.002, \mu=0, D=1$.

See Fig. 3B and C and the Supplementary Information for the derivations. First and foremost, these conditions revealed that an aggregation-inducing microbe could evolve only when its horizontal transmission rate was high enough, relative to the competing microbe (higher than a threshold that integrates the horizontal transmission rates and loss rates of both microbes). When neglecting the

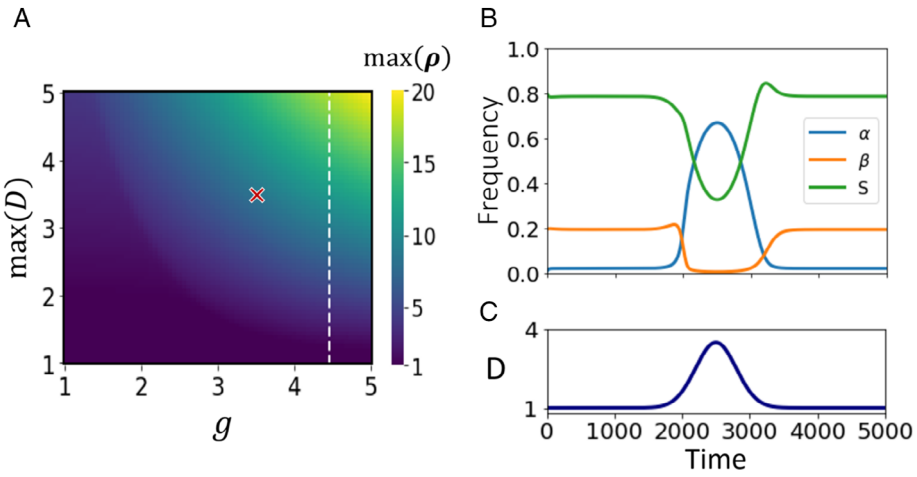

Fig 4. Temporary increase in population density enables microbe α to invade and spread under a wide range of conditions.
A. Using numerical simulations we analysed the swarming level in the population (ρ), defined as the mean number of interactions per locust: $\rho=(\beta(t)+S(t))\cdot D(t)+\alpha(t)\cdot g\cdot D(t)$. We calculated $\max_t(\rho)$ in populations that undergo a Gaussian wave of an increase followed by a decrease in its density, and plotted the maximal ρ as a function of α's induction of aggregation (g) and the population's maximal density $(\max(D))$. The area to the right of the dashed white line represents parameter values for which α reaches stable polymorphism with S also if $D=1$ throughout; while the area to the left of the dashed white line represents parameter values for which α is maintained only in mutation-selection balance proportions when $D=1$. The 'x' denotes the $\max(D)$ and g values used in panels (B–C).
B. We plotted a time-series example of the results obtained in one numerical simulation run. The figure shows the proportion of the three microbiome types along time.
C. The change in population density (D) along time. Similar density functions of time were used in all simulations, with the only difference between simulations being the maximal value of D. For panels A and B we use $T_\alpha=0.05, T_\beta=0.025, L_\alpha=0.2, L_\beta=0.02$. For panel B we also use $\max(D)=3.5, g=3.5$.

loss rate of microbes α and β, condition (5) is simplified and reveals that the aggregation-inducing microbe (α) can evolve only when it has a horizontal transmission advantage $(T_\alpha>T_\beta)$. Furthermore, we observed that within the range of conditions that allow the evolution of α, its proportion in the population increases with its horizontal transmission advantage (T_α/T_β) and with the level of aggregation it induces (g; Fig. 3). This result is consistent with the empirical evidence demonstrating that the *Weissella* genus is both associated with aggregation and indicate significant horizontal transmission ability.

Following these results, we examined the dynamics in populations that undergo both mutations and changes in their density, e.g. due to variation in the availability of resources. We modelled a population of locusts with density $D(t)$ that changes along time with a Gaussian wave (starting and ending at $D=1$). The increase in density directly affects the rate of all interactions in the population, increasing the effective horizontal transmission rate of both microbes, α and β. We denote by ρ the swarming level in the population, defined as the average interaction rate, which is affected by both the population density (D) and the fold-increase in the interaction rate of α-bearing hosts (g). We found that in this type of population dynamics, α can spread under a much wider range of conditions and contribute to the swarming. However, in many cases the invasion of α is temporary: α invades the population and spreads rapidly when the population density is high enough, generating a crowded swarm. It then declines and disappears from the population as the density decreases (Fig. 4).

Our model thus predicts that microbes, such as *Weissella* (represented by α in our model) may 'take over' the gregarious population using a combination of two effective mechanisms: the first is by enjoying a higher horizontal transmission rate than its competitors in the locust microbiome $(T_\alpha>T_\beta)$; and the second is by eliciting the conspecific interactions (g) (i.e. the conspecific attraction) of infected insects; or, within the locust context, making the individuals more gregarious (Fig. 2).

Discussion

The density-dependent phase shift of locusts from the solitary to the gregarious phenotype has been widely studied, and shown to include behavioural changes as well as physiological transitions (Pener and Yerushalmi, 1998; Pener and Simpson, 2009; Cullen *et al*., 2017; Ayali, 2019). However, the locust-bacterial composition related aspects of the phenomenon have never been directly investigated.

Locust-bacteria phase shift dynamics

The bacterium *Weissella* (Firmicutes) has been shown here to be dominant in the gregarious locust's bacterial

composition, and to be transmitted to solitary locusts upon crowding. *Weissella* members have been frequently described as dominant gut bacteria in gregarious locusts of several species, and mainly in the migratory locust (*Locusta migratoria*) (Shi *et al*., 2014; Stoops *et al*., 2016; Garofalo *et al*., 2017) and the desert locust (*S*. *gregaria*) (Lavy *et al*., 2019, 2020b, 2021). In Lavy *et al*. (2019) we reported the intriguing pattern of temporal *Weissella* blooms in the gut of laboratory-reared gregarious locusts, while the solitary locusts were shown to maintain a constant bacterial composition dominated by members of the phylum Proteobacteria. The data presented here are therefore compatible with ample previous information highlighting the presence of *Weissella* in the gut of gregarious locusts. This further demonstrates the ability of this genus to infect and dominate the microbiota of previously naive solitary locust hosts when these enter the gregarious phase. Hence this bacterium may be involved in the crowding-induced shift in the solitary locust gut bacterial composition to a gregarious-like composition, a previously unexplored aspect of locust phase transformation.

Wada-Katsumata *et al*. (2015) demonstrated that the presence of *W*. *cibaria* in cockroach faeces attracts cockroach nymphs in the vicinity and elicits their aggregation. In light of those and the present findings, although an empiric causal connection between the gregarious lifestyle and the presence of *Weissella* is yet to be established, we suggest that *Weissella* may also benefit from eliciting aggregation behaviour among locusts, thereby increasing locust conspecific encounters and thus also the bacterium's own transmission rate.

This gains support from the fact that, while being very common among gregarious locusts (Shi *et al*., 2014; Stoops *et al*., 2016; Garofalo *et al*., 2017; Lavy *et al*., 2019, 2021), this bacterium is not vertically transmitted from parent to offspring (Lavy *et al*., 2021) and thus has to rely on horizontal transmission.

In this study, we also describe for the first time the bacterial composition of the locust integument. It is shown to contain a large fraction of Proteobacteria and Firmicutes that may be partly gut-derived and a result of the dense conditions in the locust cage. We also report on a fraction of Bacteroidetes and Actinobacteria, which were not found in the faecal samples. The presence of these bacteria in both the cage of gregarious individuals and in the solitary locusts prior to their crowding supports the conclusion that these bacteria are true residents of the locust integument, and not derived from a cage effect.

Members of the Actinobacteria have been found to function as a first line of insect defence against pathogens (Kaltenpoth *et al*., 2005; Scott *et al*., 2008; Mattoso *et al*., 2012). Consequently, we hypothesize that they may also serve the same purpose in contributing to the locust's immunity, though this needs to be validated in further studies.

In both treatments, the locusts showed a significant increase in integument bacterial load at the end of the experimental period. Since this trend was common to gregarious and solitary-originated locusts, we suggest that this phenomenon might be linked to ageing or another time-dependent process of the swarm, and requires further study.

Insect aggregation behaviour can be a bacterial ecological strategy

We hypothesized that in addition to other well-established mechanisms (e.g. tactile stimuli, hormone levels and more) (Pener and Simpson, 2009; Cullen *et al*., 2017; Golov *et al*., 2018; Ayali, 2019; Knebel *et al*., 2019), the microbiome could potentially play an essential role in the locust phase shift, and that specific members of the locust microbial community might benefit from promoting crowding among gregarious populations. Although the controlled manipulations in a laboratory cage cannot, of course, fully simulate field conditions, the laboratory set-up allowed us to closely monitor individual solitary locusts following their exposure to a gregarious population and consequent phase transformation. Such a study could not be achieved in the field due to the scarcity of solitary individuals and the migration behaviour of gregarious swarms. Furthermore, both Dillon *et al*. (2008) and Lavy *et al*. (2019) have shown that the bacterial composition of laboratory locusts is broadly similar to that of field-collected individuals, suggesting that the laboratory data are ecologically relevant.

The ecological findings are also in accord with our mathematical model. Using this model, we studied the conditions that enable the evolution of an aggregation-inducing microbe (α) in a locust population. We found that a microbe benefitting from a relatively high rate of horizontal transmission may further benefit from inducing its host to aggregate and hereby increase its rate of interactions, thus enabling more transmission opportunities. This induction can be beneficial even when incurring a certain cost (represented in our model by $L_\alpha > L_\beta$). We have further shown that incorporating temporary changes in population density in the model due to external reasons allowed the aggregation-inducing microbe (α) to invade the population under a much wider range of conditions. This invasion was shown to be transient, characterized by a rapid spread of microbe α when the population becomes dense enough, and followed by a decline as population density decreases. The increase in α in the population in itself further increases the crowding,

as α-bearing locusts tend to aggregate and seek additional interactions.

A solitary-to-gregarious phase shift in the field is strongly affected by forced crowding, caused by factors such as food patchiness or a drastic population increase (Pener and Simpson, 2009; Cullen *et al*., 2017). Such conditions support and induce conspecific encounters, which potentially increase the bacterial spread within the forming swarm.

As noted, Lavy *et al*. (2019, 2020b, 2021) reported that although *Weissella* seems to spread very quickly in the gregarious population, it is not transmitted across generations, unlike other locust-associated bacteria. Our model can account for this lack of transmission across generations if interpreted as multigenerational, considering L_α and L_β, the transitions from α and β to *S*, and the lack of transitions in the opposite direction. This can represent a population in which *S* is a standard microbiome that is also transmitted across generations, while α and β cannot be transmitted or are less successful in such transmission. Therefore, a certain proportion of α and β-bearing hosts turn into *S* hosts (with rates L_α and L_β). Such failure of transmission to offspring could be due to an inability to colonize the younger life stages of the locust or due to lack of maternal inoculation (Lavy *et al*., 2021).

Concluding remarks

Using empirical experiments and a novel mathematical model, we have described here how the microbiomes of solitary individuals change upon crowding. Furthermore, we have shown that some bacterial agents can potentially benefit from the locust aggregation phenomenon, and possibly play an instrumental role in the locust density-dependent phase shift, under the controlled conditions of the laboratory. Our findings suggest that this can be beneficial for the underlying bacterium, offering it a multitude of additional potential hosts. It should be noted, however, that many questions remain unanswered; and further experiments are required in order to empirically clarify the nature of locusts–*Weissella* interaction. These should of course include the use of locust-isolated *Weissella* strains, and an in-depth exploration of the ways in which this bacterium interacts with other bacterial strains in both solitary and gregarious hosts, as well as how these interactions influence the physiology and behaviour of the locusts. Such future work will determine whether there is a causal link between the presence of *Weissella* and the aggregation behaviour, or merely a correlation driven by the opportunity for *Weissella* invasion offered by the aggregation behaviour.

This is a first description of the dynamics of the locust's microbiota while its host is undergoing the physiological and behavioural changes associated with phase transition; and our findings illuminate previously unexplored aspects of this quintessential example of environmentally induced plasticity. These findings may also open the way to potentially novel directions in the ongoing efforts to battle this ancient and devastating pest.

Experimental procedures

Insect rearing

To obtain locusts in either the solitary or the gregarious phase, locusts were reared from up to 2 h post-hatching in either solitary or crowded conditions. The gregarious and the solitary locusts were maintained in separate climate-controlled rooms, under similar ambient conditions except for density (detailed in Lavy *et al*., 2019). Briefly, light (10D: 14 L cycle) and radiant heat were supplied by electric bulbs, allowing the insect's behavioural thermoregulation between 30°C and 37°C. All locusts were fed daily with fresh wheat seedlings and dry oats.

Experimental setup

We first sampled the gut and integument bacterial composition of mature solitary-reared males and females ($t = 0$). We then transferred the locusts (marked individually with acrylic colour on their pronotum, hind femur and wings) to heavy crowding conditions, i.e. introducing them into a 65-L metal cage, containing ~200 crowded-reared locusts of similar age (simulating the locust transition from solitary conditions to swarm conditions; $t = 7$). On day 7 post-transfer, we repeated the sampling of the same individuals. Crowded-reared gregarious individuals were sampled similarly and at the same time, and used as control.

Sampling

The abdomen of mature individuals of both phases was swabbed (Heinz Herenz, Hamburg, Germany), followed by inserting each locust, head first, into a sterile 50 ml centrifuge tube (Corning, NY, USA) for 3 h, to collect its faecal pellets. This setup prevented the insects from moving and ensured the collection of uncontaminated faeces from each individual. Swab samples and the faecal pellets were marked as '$t = 0$' and kept at −80°C until DNA extraction. Re-sampling at day 7 was conducted in the same manner; samples were marked '$t = 7$' and stored at −80°C until further use.

DNA extraction and sequencing

Bacterial DNA was extracted using the 'Powersoil' DNA isolation kit (Mo Bio Laboratories, Carlsbad CA, USA)

following the manufacturer's instructions, using 60 μl for final DNA elution. To determine the bacterial composition of the different samples, a polymerase chain reaction (PCR) was applied with universal primers 5′ end common sequences (CS1-341F 5′-ACACTGACGACATGGTTCT ACANNNNCCTACGGGAGGC AGCAG and CS2-806R 5′-TACGGTAGCAGAGACTTGG TCTGGACTACHVGGG TW TCTAAT), amplifying the variable regions 3 and 4 of the prokaryotic 16S rRNA gene. The PCR conditions were one cycle of 95°C for 3 min, followed by 31 cycles of 95°C 15 s, 55°C 15 s, 72°C 5 s; using the PCR master mix KAPA2G Fast™ (KAPA Biosystems, Wilmington MA, USA). Amplified products were visually verified and sent for deep sequencing on an Illumina MiSeq platform at the Chicago Sequencing Center of the University of Illinois.

DNA was also extracted according to the protocol above from two clean swab sticks as a control. Visual validation showed no PCR product in these samples.

Estimation of bacterial absolute abundances

Absolute amounts of bacterial DNA per sample were estimated using QPCR as presented in Contijoch *et al*. (2019). 1 μl DNA was added to PCRBIOs SyGreen master mix, and underwent 40 PCR cycles (15′ 95C, 15′ 53C, 10′ 72C) run (BIORAD CFXConnect RT system) using the following 16S primers: 515F-5′-GTGC CAGCMGCCGCGGTAA, 806R-5′-TACGGTAGCAGAGA CTTGG TCTGGACTACHVGGGTW TCTAAT. Three replicate wells were loaded per sample, with two no template controls and a reference standard (serial dilutions of ZymoBIOMICS© Microbial Community DNA Standard, spanning 0.0016–0.6 ngDNA μl^{-1}) loaded on each plate. DNA concentration per sample was calculated by the formula: DNA Concentration $= \exp(\alpha \times Cq + \beta)$, where α and β are the slope and intercept coefficients derived from the standard curve loaded on that same plate. Finally, to translate relative bacterial abundances to absolute ones, a custom R script was used to multiply the relative abundances of each sample by its absolute bacterial load.

Data analyses

De-multiplexed raw sequences were merged using PEAR (Zhang *et al*. 2014), discarding sequences of less than 380 bp, and sequencing primers removed using the 'extract_barcodes.py' script from Qiime1 (Caporaso *et al*. 2010) (SRA archive accession number: PRJNA745394). Data were then processed with the DADA2 pipeline (Callahan *et al*. 2016) to infer exact sequences for ASV analyses, using the SILVA database (version 128) as reference for taxonomic assignment. Sequences identified as Chloroplast (originated in the locusts diet) were removed from the data using base R subsetting operators. Sample depth post-processing ranged from 4 to max 29 051 seqs/sample; to ensure data evenness, before analysis the data were rarified to 2500 seqs/sample. All statistical analyses were conducted using 'R' v.3.4.1. (R Core Team, 2020). Rarefaction, canonical analysis of principal coordinates and analysis of similarities were carried out using Vegan (Oksanen *et al*., 2008, function used: 'capscale'). Kruskal–Wallis rank-sum test was conducted using the 'stats' package (base R), and Dunn's test was conducted using the 'dunn.test' package (Dinno, 2017).

Mathematical model description

We modelled a fully mixed population of locusts, each bearing one of three microbiome compositions, and used a compartmental model that describes the change in the frequencies of the different microbiome compositions in the population over time. Denoted by S are locusts bearing a standard solitary microbiome composition. Denoted by α are locusts bearing, in addition to the standard microbiome, also microbes of type α that induce their hosts to aggregate, namely, to initiate more interactions. We denote by $g \geq 1$ the factor that controls the fold-increase in the rate of interactions of locusts bearing microbes of type α. Denoted by β are locusts bearing, in addition to the standard microbiome, also microbes of type β, which do not affect their host's behaviour.

During interactions between locusts, microbes α and β can be transmitted from one locust to another with probabilities T_α, T_β. We assume that α and β cannot co-reside within one host, and thus upon interactions between an α-carrying host and a β-carrying host, transmission of the α microbes would result in replacement of the residing β microbes with the transmitted microbes, and vice versa for β to α transmission. We assume that the hosts bearing α/β microbes can restore their standard microbiome and lose α/β at the corresponding rates L_α, L_β. We focus here on the cases in which α microbes have a higher transmission rate than β microbes $(T_\alpha > T_\beta)$, and a lower persistence within the microbiome $(L_\alpha > L_\beta)$.

We further account for increase and decrease in *all* rates of interactions due to changes in population density, denoted by D. Finally, we include low rates of mutation in all directions: $S \leftrightarrow \alpha$, $S \leftrightarrow \beta$ and $\alpha \leftrightarrow \beta$. For simplicity, we assume a constant mutation rate of μ in all directions.

The following three differential equations represent the dynamics in the population (see Fig. 3A and Supplementary Information for analysis of the system):

$$\frac{d\alpha}{dt} = \alpha \cdot S \cdot \mathrm{D} \cdot g \cdot T_\alpha + \alpha \cdot \beta \cdot \mathrm{D} \cdot g \cdot \left(T_\alpha - T_\beta\right) - \alpha \cdot L_\alpha + \mu \cdot (S + \beta - 2 \cdot \alpha) \tag{E1}$$

$$\frac{d\beta}{dt} = \beta \cdot S \cdot \mathrm{D} \cdot T_\beta + \beta \cdot \alpha \cdot \mathrm{D} \cdot g \cdot \left(T_\beta - T_\alpha\right) - \beta \cdot L_\beta + \mu \cdot (\alpha + S - 2 \cdot \beta) \tag{E2}$$

$$\frac{dS}{dt} = -S \cdot \alpha \cdot \mathrm{D} \cdot g \cdot T_\alpha - S \cdot \beta \cdot \mathrm{D} \cdot T_\beta + \alpha \cdot L_\alpha + \beta \cdot L_\beta + \mu \cdot (\alpha + \beta - 2 \cdot S) \tag{E3}$$

where $\alpha + \beta + S = 1$

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1: Supplementary Information.

Fig. S1. Absolute amounts of bacterial DNA per sample in the faeces **(a)** and the integument **(b)** of gregarious-grown (yellow underscore) and solitary-grown (green underscore) individuals at t = 0 d and at t = 7 d, where t = 0 d is the time when the solitary-grown locusts were moved to crowding conditions. Pairwise comparisons between the groups were conducted using Wilcoxon Rank Sum test.