

A network of sex and competition: The promiscuous mating system of an invasive weevil

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Abstract Invasive alien pest insect species represent a major threat for agriculture and biodiversity. Because chemical treatments employed to contrast such pests elicit serious environmental and human health problems, a great effort is currently directed to develop long term and environmentally friendly biological control strategies. However, the successful application of some promising techniques, such as the Sterile Insect Technique (SIT), requires a deep knowledge of the pest basic biology. Here, we argue that understanding pest sexual biology using a social network approach can significantly improve the performance of control strategies. For example, SIT may benefit from understanding how individuals interact and how males accede to reproduction, in order to target the most reproductively active and polygamic males. In this paper we studied the socio-sexual networks of the Asian red palm weevil (RPW) *Rhynchophorus ferrugineus*, a worldwide invader which is causing heavy economic impacts on several palm species. We found that the RPW has a highly promiscuous mating system, characterized by forced interruptions of pair copulations by additional males. The social network is highly non-random nor regular: few males almost monopolize reproduction, behaving as key-players in the network of matings. Additionally, males have a stable pattern of sexual behaviour over time. We use RPW social network as a case study to direct the development of management techniques such as SIT strategy [Current Zoology 61 (1): 85–97, 2015].

Keywords Social network analysis, Pest management, Mating system, Reproductive skew, Alien pest, *Rhynchophorus ferrugineus*

Invasive alien species (hereafter IAS) represent one of the major threats for biodiversity, human health and food production worldwide (Kettunen et al., 2008; Hulme et al., 2009; Mazza et al., 2014a). Insects play a major role in biological invasions and impose dramatic environmental and economic impacts (Kenis et al., 2009). As an example, one of the latest arrived pests in Western countries, the small fruit fly *Drosophila suzukii*, is estimated to cost more than 500 million dollars every year only in the USA (Bolda et al., 2010; Cini et al., 2012). After decades of pesticide-based approach, pest management is now moving to more environmentally friendly approaches, such as increasing IAS mortality by using biocontrol agents (i.e. fungi, bacteria, viruses and other natural enemies of the pest, such as predators and parasitoids) or reducing IAS reproduction through interferences with their communication systems (e.g. the chemical one, Nordlund et al., 1981; Witzgall et al., 2010; or the vibrational one, Eriksson et al., 2012).

Pest reproduction control is also possible through the

‘sterile insect technique’ (hereafter SIT), i.e. the massive release of sterile insects to reduce fertility of a wild population of the same species (Dyck et al., 2005; Klassen and Curtis, 2005). The development of the SIT represented a major breakthrough in pest management science (Vreysen et al., 2006). Indeed, SIT has several advantages compared to other control strategies, e.g. being safe to non-target organisms and acting with an inverse density-dependent mechanism; in addition, SIT could be integrated with other control strategies (Vreysen et al., 2006) and it has been used with success especially against Diptera, Coleoptera and Lepidoptera (Bakri et al., 2005).

Compared to traditional control methods based on the insecticide application, the use of biological control strategies requires knowledge of the basic biology of the pest, in order to obtain efficacy in both pest reduction and economic terms. The success of the SIT, in particular, requires that sterile males show similar competitiveness, survival and dispersal ability as wild males.

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Moreover, sterile males should be able to mix with the wild population and wild females should show no discrimination against captive-reared sterile males. A key step to assess whether sterile males meet these relevant ecological and behavioural requirements is gaining a deep understanding of the basic reproductive biology and especially the sexual behaviour of the target pest species in a natural context (Hendrichs et al., 2002).

In this paper we studied an invasive alien pest, the Asian red palm weevil *Rhynchophorus ferrugineus* (Olivier, 1790) (Coleoptera: Dryophthoridae) (hereafter abbreviated as RPW) to understand how its basic reproductive biology may affect the successful application of the SIT. RPW has recently invaded several countries of southern Europe and has rapidly become the worst pest of ornamental palms (reviewed in Giblin-Davis et al., 2013). Adults of RPW are attracted by dying or damaged palms where mating occurs and where inseminated females lay eggs. Larvae feed on soft central tissue of the palm, causing the palm to collapse and die (Wattanapongsiri, 1966; Giblin-Davis et al., 1996; Murphy and Briscoe, 1999). RPW's invasive potential is due to high female fecundity (which results in several generations per year even in the same tree, Esteban-Duran et al. 1998; Faleiro 2006), long dispersal distances of adults, and the wide host range (at least 20 species of palm have been reported as hosts worldwide; Giblin-Davis et al., 2013).

Due to the threats RPW poses to human economy, several studies have been carried out on how to manage this species (Giblin-Davis et al., 2013; Mazza et al., 2014b). Management of RPW is difficult due to the cryptic habits of both adults and immature brood. Although application of large quantities of synthetic insecticides is generally used to limit the palms infestation, several alternative environmentally friendly solutions based on biological control are under study (reviewed in Mazza et al., 2014b). Recent studies have been aimed at assessing the efficacy of the sterile male release technique, as this species is easily mass reared in laboratory conditions (Faleiro, 2006), thus satisfying a crucial aspect for the feasibility of the SIT. However, results are not exhaustive enough to allow its broad application (e.g. Rahalkar et al., 1973; Krishnakumar and Maheswari, 2007; Al-Ayedh and Rasool, 2010; Llàcer et al., 2013; Paoli et al., 2014).

Unfortunately, information on the reproductive behaviour in the RPW is scant (Kaakeh, 2005): mating is reported to occur on dying or damaged palms which beetles are attracted to also thanks to the male released

aggregation pheromone (Hallett et al., 1993) whose most important compounds are ferrugineol (4-methyl-5-nonanol) and ferrugineone (4-methyl-5-nonanone) which have effects on both sexes and induce adults aggregation (Gunawardena and Bandarage, 1995; Poorjavad et al., 2009). The most information on RPW mating system (frequency, duration, behavioural pattern) comes from laboratory studies in which adult males and females are kept in single pairs, to better monitor particular behaviours such as oviposition and larval development (e.g. Gothi et al., 2007; Prabhu et al., 2010; Ince et al., 2011). These procedures, despite allowing the rearing and study of a great number of animals, can be misleading if used to infer the RPW behaviour in wild situations.

As SIT is based upon the interference with the pest's mating system, a deep knowledge of the sexual and social behaviour of RPW has the potential to improve the development and the tuning of the SIT. Here, we provide the first description of the behaviour of RPW *Rhynchophorus ferrugineus* in aggregation context, performing laboratory behavioural observations using mixed groups of RPW (as it occurs in the wild), with the aim of reproducing as close as possible the situation found inside and on the palms, where interactions and matings take place (Giblin-Davis et al., 2013).

We first describe the socio-sexual network of groups of RPW, unravelling the behaviours involved and the role played by males and females in the mating context. We then focus on the sexual activity of males (the targets of SIT) and adopt a social network analysis approach to understand whether males differ in their reproductive effort (i.e. the number of mating acts performed by an individual) and in the number of partners they mate with; these are two traits that likely affect males reproductive success (e.g. Keller and Reeve, 1995; Campbell, 2005; Abdel-Azim et al., 2012), being thus of great interest for the application of the SIT. Additionally, we evaluate whether these traits are stable over time and whether they are linked to size.

Every individual is part of a network of inter-individual associations which vary in strength, type and dynamics (Croft et al., 2008). The structure of this social network has broad consequences to individual fitness. Social network analysis (SNA) provides a conceptual and analytical framework to uncover the structure and function of simple and complex social networks, and gained in the last decades a pivotal role in depicting the functioning of many animal social systems, from the solitary (e.g. Mourier et al., 2012) to very complex primate and insect societies (e.g. Sueur et al.,

2011; Mersch et al., 2013; Baracchi and Cini, 2014; Pinter-Wollman et al., 2014). In our paper we used social network analysis to investigate the socio-sexual groups of an invasive pest beetle with the aim of improving the application of environmentally friendly management techniques.

1 Materials and Methods

1.1 Animal collection and rearing

We collected 136 RPW adults (68 males and 68 females) in September 2010 and 2011 from two sites in Sicily (Catania and Trapani provinces), using the same methods as in Mazza et al. (2011), both from traps baited with aggregation pheromone and from infested palms (untreated specimens of *Phoenix canariensis*, one of the main host in the Mediterranean basin, Giblin-Davis et al., 2013). The RPW were sexed (according to Wattanapongsiri, 1966) and males' size was measured (total length from the top of the rostrum to the end of pygidium) using the software IMAGEJ (Rasband 1997–2012). Total length was chosen as it is highly positively correlated with other morphological measures (pronotum length, pronotum width, abdomen length and total weight, $r > 0.518$, $P < 0.001$, $n = 40$) and is the easiest and most rapid to measure. Each weevil was individually marked on the pronotum, and kept isolated in a small plastic box ($15 \times 15 \times 15$ cm) under stable laboratory condition (temperature: $22 \pm 2^\circ\text{C}$, Humidity: 50%, natural L:D cycle regime) and fed *ad libitum* with apple slices, until behavioural observation began.

1.2 Behavioural observation

In order to describe the RPW mating system we observed socio-sexual interactions in three mixed sex groups (see below, groups 1–3), while a fourth group was used to assess stability of sexual behaviour over time (see below). The weevils were kept in different arenas according to their collection site: Groups 1 and 2, hereafter G1 ($n = 36$) and G2 ($n = 24$), from Trapani, group 3 and group 4, hereafter G3 ($n = 36$) and G4 ($n = 40$), from Catania. The sex ratio of each group was 1:1, which simulated the sex ratio recorded in the wild (Longo et al., 2009). Observation sessions were performed in September 2010 (G1–G3) and 2011 (G4) by placing individuals of both sexes in a glass arena without shelter ($45 \times 25 \times 27$ cm).

Behavioural observations were performed by two researchers during the day (between 10 a.m. and 5 p.m.) when RPW are active (Fanini et al., 2014) under the same laboratory conditions as maintenance. Preliminary observation suggested that overall activity levels can vary among groups. In order to obtain a representative

and comparable picture of the social network of each group, we thus performed behavioural observations until at least 90% of nodes entered the network and at least 50% of links were resampled. Because interaction rate was different across groups, this resulted in a different duration of observations for each group (between 80–160 minutes, see Table 1). Preliminary observations also suggested that sexual behaviour in group was frenetic and that immediately after a mating effort, the weevils looked for another partner to mate with. In such a promiscuous environment, matings appeared as males' coarse attempts to mate with inconspicuous "ritualized" rostral rubbing, antennal tapping, or mate guarding as reported by Al-Ayedh and Rasool (2010). For this reason, we decided to focus on the most evident behavioural patterns, and we recorded during each session (all occurrences sampling, Altman (1974) the occurrence of: the number of (1) male-female mating (2) male-male mating and (3) forced mating interruption and recorded the actor and receiver of each interaction. Matings were deemed to occur when the aedeagus was inserted without any assumption about spermatophore transfer (Vanderbilt et al., 1998). By forced mating interruptions we refer to the interactions between a couple (both male-male and male-female) that were interrupted by the intromission of the rostrum by a third weevil, as observed also by Vanderbilt et al. (1998) in another weevil species, *R. cruentatus*. For forced mating interruptions we consider the interaction as occurring between the individual who performed the interruption (actor) and the male (receiver) who was performing the mating (either with a female or a male). We first used all interaction types to describe the overall interaction pattern in the sexual contexts, and then focused our analysis on the reproductive interaction (the interest of the SIT), thus using only heterosexual interactions. Two traits can be of interest to evaluate individual mating success in males: the reproductive effort (i.e. the number of matings performed by an individual) and the number of partners of a specific male (outdegree). The two traits may independently affect male reproductive success (e.g. Keller and Reeve, 1995; Campbell, 2005; Abdel-Azim et al., 2012) and have been thus considered separately.

Preliminary observations carried out in the wild suggested that the behavioural patterns we recorded are not artefacts due to laboratory conditions: in the field the same three types of interactions were observed with similar frequencies and visually inspection of the socio-sexual networks suggested a similar structure (authors' personal observation).

Table 1 Descriptive data on RPW interaction network

Group (males: females)	G1 (18:18)	G2 (12:12)	G3 (18:18)
Observation time (minutes)	160	80	150
Interactions observed	148	146	62
% Heterosexual matings	60.8	60.3	67.7
% Homosexual matings	25.7	28.8	12.9
% interruptions	13.5	11.3	19.4
Nodes	36	24	36
Links	73	96	45
Isolates (Males:females)	5 (2:3)	0	6 (2:4)
Activity distribution different from Poisson distribution?	$Z = 0.2383, P < 0.001$	$Z = 1.517, P < 0.040$	$Z = 1.532, P = 0.003$
Degree distribution different from Poisson distribution?	$Z = 1.532, P = 0.006$	$Z = 0.859, P < 0.222$	$Z = 1.135, P = 0.039$
Indegree distribution different from Poisson distribution?	$Z = 0.348, P = 0.852$	$Z = 0.554, P = 0.589$	$Z = 0.799, P < 0.174$
Outdegree distribution different from Poisson distribution?	$Z = 3.210, P < 0.001$	$Z = 2.360, P < 0.001$	$Z = 1.614, P = 0.002$

Stability of males' sexual behaviour (mating effort and number of partners) over time was assessed using G4, which was observed at three time points: the day the group was formed (day 1), three days later (day 4) and nine days later (day 10), each time for two hours. This time frame was chosen because field and laboratory observations suggested that group composition is dynamic, interaction rates rapidly fall after the first days, and sterilization drastically reduces male survival (authors pers. observation and M. Cristofaro pers. communication). Between observation trials individuals were maintained in the group and fed with apple slices. Because we were interested in testing whether male sexual behaviour was constant over time, we focused only on heterosexual matings, recording the actor (i.e. the individual that begins the interaction) and the receiver of the interaction.

1.3 Social network analysis and statistical analysis

Social networks for each group were built based on the pairwise interaction data: each individual is a node and a link is considered existing between two nodes when at least one interaction occurred between them. In order to provide a general description of the social interaction network we calculated for each individual the level of activity (number of performed interactions) and we choose degree (number of nodes with whom a focal node has one link at least) as a centrality measure. The same measures were subsequently calculated for males only considering heterosexual interactions: in this case, the number of interactions performed represents the reproductive effort and the degree represents the number of partners. (As mating and interruption are asymmetrical actor-receiver interactions, links are oriented from the initiator to the receiver of the interaction. Directed

centrality measures (outdegree, i.e. the number of different nodes towards which a focal nodes performed at least one interaction; indegree, i.e. the number of different nodes from which a focal node received at least one interaction) were thus calculated. In order to separate activity levels from centrality we calculated unweighted centrality measures. Networks were visualized using the Spring embedding technique as implemented in Netdraw (Borgatti, 2002).

In order to obtain a representative sample of the overall population and to perform a population level analysis with increased statistical power we pooled data from the three groups. The slight differences in group size and group overall activity did not affect individual behaviour, allowing us to use raw data in the pooled sample. Data related to each of the three groups can be found throughout the text and in Supplementary materials.

Distributions of activity levels and centrality values were compared with a Poisson distribution using Shapiro-Wilk test. Mann Whitney test with Montecarlo randomization procedure was used to compare activity and centrality levels between genders. Quadratic assignment procedure (QAP) was used to compare matrices built using different interactions (all interactions vs only heterosexual matings) and at different time periods (heterosexual network of G4 on days 1, 4 and 10). In order to compare male reproductive effort and number of partners at different time points we performed a repeated measure ANOVA with post-hoc Sidak corrected pairwise comparisons (Field, 2005) on log-transformed data. Sphericity assumption was met in both cases (Mauchly's test, $\chi^2 = 0.056, df = 2, P = 0.972$; $\chi^2 = 0.076, df = 2, P = 0.963$). Pearson correlation was used to compare reproductive effort and number of partners

among them. Furthermore, the relationship between these variables and individual length was explored. In order to avoid alpha inflation due to multiple comparisons, significant results have been compared to significance threshold calculated according to the False Discovery Rate procedure (Benjamini and Hochberg, 1995). Statistical analyses were performed using Ucinet (Borgatti et al., 2002), SPSS and Past software (Hammer et al., 2001).

2 Results

2.1 General description of RPW social network

RPW were highly active in all groups: 0.41 to 0.98 interactions were recorded per minute. The frequency of the behaviours observed, i.e. heterosexual mating, homosexual mating and mating interruption, was similar among the three groups ($\chi^2 = 9.29$, $df = 4$, $P = 0.054$, the tendency to be significant is due to aggregation in G3, where less homosexual matings and more interruptions were observed compared with the other two aggregations, Table 1). Heterosexual matings represented the most frequently performed interactions (between 80.6 and 89.1% of all interactions), representing about two thirds of the total interactions. Homosexual matings occurred at a rate of 12.9 to 28.8 % (Table 1). Forced interruptions represented 11.3–19.4% of the observed interactions and are described here for the first time in the RPW. These interactions consisted of a male separating a mating couple (either a male–female couple or a male–male couple) by inserting its rostrum between the two weevils and using it as a crowbar to coerce them to interrupt copulation.

RPW interaction network in the mating context is highly promiscuous: each individual interacted with many other individuals (Fig. 1 reports the interaction network for one aggregation, G1, networks of G2 and G3 are reported in Fig. S1 in Supplementary Materials). SNA reveals that the socio-sexual network is composed of one component, with no identifiable cliques. In two networks a few individuals (Table 1) were never observed interacting (neither actively nor passively) with any other individual, so some isolates occurred.

Overall, individual behaviour and network properties were highly variable among individuals: both activity and degree distributions were positively skewed and different from a Poisson distribution (activity: $Z = 3.351$, $P < 0.001$, $n = 96$; degree: $Z = 2.450$, $P < 0.001$, $n = 96$, Fig. S2). These findings suggest the existence of a small fraction of highly active and highly connected individuals together with a large fraction of poorly

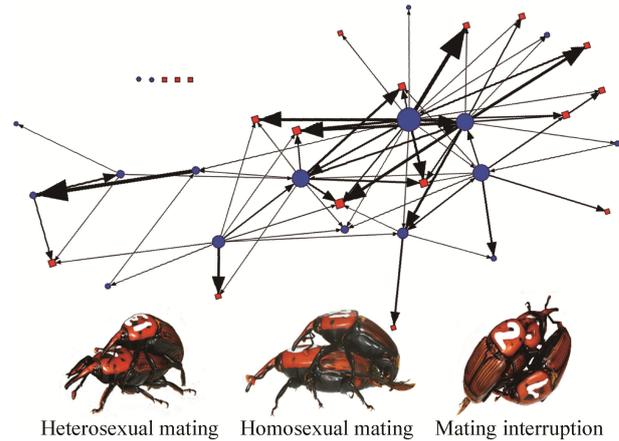


Fig.1 The promiscuous socio-sexual network of RPW in mating aggregations

Interaction network of RPW G1, visualized with the Spring embedding technique. The three behaviours recorded were heterosexual mating, homosexual mating and forced mating interruption. Nodes represent individual weevils and edges represent interactions. Node size is proportional to degree (number of nodes with which the focal nodes had at least one interaction). Edges thickness is proportional to edge strength (number of interactions occurred, pooled for the three behaviours) and arrows point toward receivers. Unconnected circles represent individuals that never did nor received an interaction (isolates). Blue circles: males, red squares: females.

active and poorly connected individuals. Indeed, in the overall population, 20% of RPW is responsible for 80% of the socio-sexual interactions (range for single groups: 13%–33%, Fig. S2).

2.2 Different activities and social network positions between genders

Males and females showed striking differences in their behaviour. While males were engaged in high frequencies of sexual and interruption behaviours, females never started an interaction, they were never observed soliciting an interaction nor interrupting other couples (while they were nonetheless active and moving around). Consequently, males have, on average, higher values of networks measures than females (degree: Mann Whitney test, $U = 864.0$, $P = 0.033$, $n = 48$ vs 48; out-degree: Mann Whitney test, $U = 192.0$, $P < 0.001$, $n = 48$ vs 48, Fig. 2) except for incoming centrality, for which females show higher values (indegree: Mann Whitney test, $U = 852.0$, $P = 0.029$; $n = 48$ vs 48, Fig. 2).

Interestingly, males were also more variable in their centrality measures than females, except for the incoming degree (numbers of individuals from whom a focal individuals received at least one interaction), which is similar between gender (interquartile range for males: degree = 9.75; outdegree = 11.75; indegree = 5.0; for females: degree = indegree = 7.0, out degree not available

as females never started an interaction). Activity levels and outdegree were positively correlated in males (Pearson $r = 0.978$, $P < 0.001$, $n = 48$). Interactions performed and received, as well as outdegree and indegree were positively correlated in males (respectively Pearson $r = 0.531$, $P < 0.001$, $n = 48$; Pearson $r = 0.508$, $P < 0.001$, $n = 48$): more active males were also the ones who received more interactions and males who interacted with many partners were also the ones who received interactions from many partners.

2.3 Focus on male sexual behaviour

Social network structure of male sexual behaviour (considering only heterosexual matings) mirrors that of all interactions (heterosexual, homosexual mating and mating interruption). Indeed, the networks of sexual interactions are positively correlated with the networks of all interactions (QAP, G1: $r = 0.789$, $P < 0.001$; G2: $r = 0.560$, $P < 0.001$; G3: $r = 0.819$, $P < 0.001$).

Male reproductive effort was highly variable (Fig. 3 reports the network for G1; networks of G2 and G3 are reported in Fig. S3). Within each group, males show a wide range of variation both in the relative reproductive effort (range: G1 = 0–32; G2 = 0–18; G3 = 0–8) and in their number of partners (range G1 = 0–11; G2 = 0–10, G3 = 0–5). In all groups, the distribution of male reproductive effort is highly positively skewed, with few males performing many matings and many males performing few matings. Similarly, also the distribution of the number of partners is highly positively skewed, with few males mating with many females while the largest

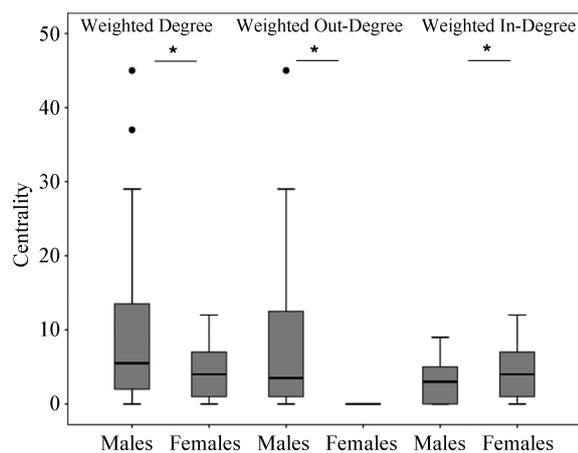


Fig.2 Males and females show striking differences in their behaviour

Box plots show the degree, outdegree and indegree of males and females. Boxes represent the 25th–75th percentiles, middle lines represent the median, whiskers represent the highest and lowest values. Circles report outliers. * indicates significant comparisons (details in the text).

part mates with a few or even no females. Overall, few males in the population are responsible for the largest part of sexual interactions, either when considering reproductive effort than when considering numbers of females with whom males mated: considering the whole population, 18% of males are responsible for 80% of observed links, and 30% of males are responsible for 80% of mating acts (data for separated groups: % of males responsible for, respectively, 80% of mating acts and 80% of observed links: G1:22%, 22%; G2: 11%, 58%; G3: 22%, 33%). As a consequence, when considering the whole male population, male reproductive effort and number of partners are highly positively skewed, thus not following the prediction of a random Poisson distribution (Kolmogorov-Smirnov test, reproductive effort: $Z = 2.636$, $P = 0.008$; number of partners: $Z = 2.075$, $P < 0.001$, Fig. S4). When restraining analysis within each group, the same non-random distribution holds in two out of three groups for reproductive effort distribution (G1, $Z = 2.421$, $P < 0.001$; G2, $Z = 0.653$, $P = 0.466$; G3, $Z = 1.238$, $P = 0.024$, Fig. S5–S7, Supplementary Materials) and in one out of three groups for number of partners distribution (G1, $Z = 1.843$, $P < 0.001$; G2, $Z = 0.585$, $P = 0.565$; G3, $Z = 0.892$, $P = 0.107$, Fig. S5–S7, Supplementary Materials).

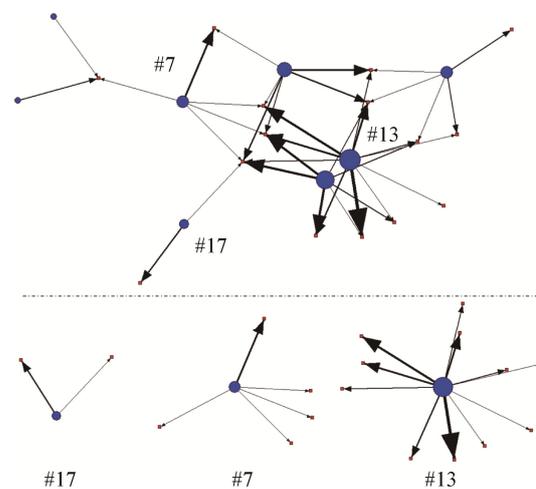


Fig.3 Males show a large variability in their sexual behaviour

Sexual interaction network of RPW G1, visualized with the Spring embedding technique. Nodes represent individual weevils and edges represent interactions. Node size is proportional to the number of partners (number of nodes toward which the focal nodes performed at least one interaction). Edges thickness is proportional to edge strength (number of interactions occurred, only heterosexual mating were considered) and arrows point toward receivers. Numbers refer to individual identity. Isolates are not reported. Blue circles: males, red squares: females. Below the dashed line the ego-networks of three males with very different reproductive effort and number of partners are reported.

Reproductive effort was highly correlated with numbers of partners with whom an individual mated at least once ($r = 0.985$, $P < 0.001$, $n = 48$, Fig. 4): more sexually active males also had more partners. Male size was not correlated with sexual behaviour neither considering reproductive effort (Pearson $r = -0.079$, $P = 0.592$, $n = 48$, Fig. 4) nor considering number of partners (Pearson $r = -0.094$, $P = 0.523$, Fig. 4). Indeed, male size was not correlated even with individual overall activity (considering the three behavioural items, size \times number of interactions performed: Pearson $r = -0.087$, $P = 0.556$, $n = 48$).

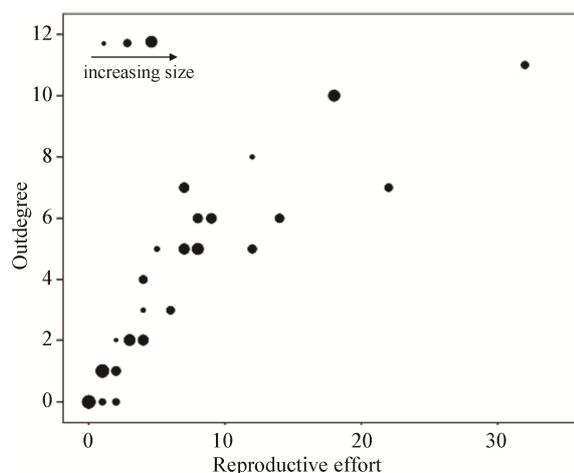


Fig.4 More active males are also the more polygamic ones, but not the bigger ones

Scatter dot of the relationship between reproductive effort and number of partners (outdegree) in males of the overall population. Circle size is proportional to individual length.

Sexual activity of males significantly decreases across time (Fig. S8) both for reproductive effort (Repeated measure ANOVA, $F = 21.106$, $df = 2$, $P < 0.001$) and for number of partners (Repeated measure ANOVA, $F = 17.689$, $df = 2$, $P < 0.001$). Males were more sexually active in the first observation period than in the second (Post hoc pairwise comparison, for both reproductive effort and numbers of partners $P < 0.001$) and the third ones (Post hoc pairwise comparison, for both reproductive effort and numbers of partners $P < 0.001$) but no difference was observed between the second and the third period (Post hoc pairwise comparison, reproductive effort $P = 0.998$, number of partners $P = 0.994$). Sexual network structure was maintained across time, i.e. matrices of interactions at different time period were positively correlated (Table 2). Accordingly, individual reproductive effort was not positively correlated among the first observation period and the others, while it was between the second and the third period (only a non

significant, positive trend however remained after correcting for multiple comparisons, Table 2). Number of partners was not correlated between day 1 and day 4, nor between day 1 and day 10, while a positive trend was observed between day 4 and 10 (Table 2).

Table 2 Stability of heterosexual network structure and individual sexual behaviour over time

	Comparison	Correlation	Significance
Overall network structure	t1 vs t2	0.279	<0.001
	t1 vs t3	0.201	<0.001
	t2 vs t3	0.152	<0.001
Males reproductive effort	t1 vs t2	0.239	=0.310
	t1 vs t3	0.264	=0.261
	t2 vs t3	0.491	=0.028
Males number of partners	t1 vs t2	0.251	=0.285
	t1 vs t3	0.176	=0.459
	t2 vs t3	0.352	=0.129

3 Discussion

Our study provides the first description of the mating system and the social-sexual interactions in a gregarious context of *Rhynchophorus ferrugineus*, the major pest of palm trees. Our observations allowed us to depict a complex network of sexual (hetero and homosexual) and competitive (mating interruption) interactions, characterized by several relevant features which were so far unknown for RPW.

First, we highlighted that in a gregarious context both homosexual matings and mating interruptions occur at a relevant, non-anecdotal rate. Both interactions were not known for RPW and showed similarity in their pattern with observations made on a related species, *Rhynchophorus cruentatus* (Vanderbilt et al., 1998). Even if *ad hoc* studies are required to disentangle the many possible explanations for these behaviours (such as social dominance, sexual training and sperm transfer for homosexuality, Levan et al., 2009), our data advocate they may play a relevant role in RPW reproduction.

A striking feature of RPW mating aggregations is the high level of interconnections between individuals. Males, in particular, performed frenetic searches for a partner seemingly without a specific preference, rapidly mating with many partners. This overexcited activity is particularly evident when individuals are first put together (day 1) and decreases later, stabilizing on the fourth day. This “frenetic promiscuity” could be elicited by the increased level of aggregation pheromone, which is known to play a role in the stimulation of sexual activity,

in such a crowded situation, as pointed out by Vanderbilt et al. (1998) for the congeneric *R. cruentatus*. The aggregation pheromone attracts “ready-to-mate” adults and already fecundated females (Gunawardena and Bandarage, 1995; Kalleshwaraswamy et al., 2005; Poorjavat et al., 2009).

The strong promiscuity we found, however, does not imply inter-individual homogeneity of behaviour. Indeed, we found large variability both in the individual activity levels and in the individual centrality: few individuals were highly active and connected inside the interaction network, while most of the individuals were poorly active and poorly connected. This variability can be, at least partially, explained by differences between and within gender. Indeed, males and females show striking differences. Males have, on average, an active role in the interaction network: they look for matings and they interfere with rivals, showing, on average, higher levels of activity and higher levels of centrality. Females, on the other hand, never started an interaction adopting a passive role. As homosexual matings were quite common, males receive quite a few interactions, resulting in having similar levels of incoming connections compared with females.

Male behaviour was highly variable, both considering all interactions and only heterosexual behaviour (which is the focus of SIT). Few males seem to behave as hubs in the networks, performing more acts and being more connected than others. Regarding heterosexual matings only, some males can be up to six times more active and have four times more partners (in approximately two hours of observation) than the average. The positive correlation between males' reproductive effort and the numbers of partners they interacted with suggests that differences among males are linked to overall levels of activity rather than to specific strategies.

Our findings offer interesting opportunities for the application of SIT. SIT involves the mass rearing, sterilizing and releasing of large numbers of insects to reduce or eliminate subsequent generations of populations of the target pest. Usually, these released sterilized males are taken without selection from the reared set (Dyck et al., 2005). Our findings suggest that a selection of the more sexually active males for sterilization may strongly enhance the efficacy of SIT. Male reproductive success is affected, amongst other things, by two main traits: reproductive effort (i.e. the number of matings performed by an individual) and the number of partners. These two traits may have relevant consequences on the application for the SIT. First, two males may have

similar reproductive effort but rather different numbers of partners. Under a SIT perspective, the sterilization of a male with many matings but with only few partners and the sterilization of a male with few matings but many partners will have very different effects on population fecundity. Indeed, sterilization of the latter one will impair reproduction of many more females than the former. Then, SIT may be more effective where, at the population level, access to reproduction is monopolized by a few males than where it is more evenly distributed (provided that SIT manages to target the hub males).

The level of heterogeneity of these traits that we found inside our study populations should thus be taken into account for future RPW population control through the release of selected sterile males. Indeed, a few males (around 20%) almost monopolize reproduction, being responsible for 80% of matings and mating with the 80% of females in the population. Interestingly, males maintain their behavioural performance over time, suggesting that targeting these males may result in a stable impairment of population reduction of a greater magnitude compared to a sterilization of randomly chosen males.

SNA represent a strong tool for identifying the best targets, but unfortunately it requires a large sampling effort, thus reducing the feasibility of this approach for the SIT. The selection of more active and connected males for sterilization could thus benefit from finding a phenotypic correlate of the male sexual activity. In our study, size, the most evident and variable phenotypic trait potentially involved in the competitive gregarious context of RPW mating, it is not correlated with males' sexual activity. Future studies are needed to examine whether other intrinsic factors such as age (even if mating success is not influenced by age in a Spanish population, Llacer et al., 2013), reproductive status, hormone titres or behavioural syndromes may affect males sexual behaviour (Hanks et al., 1996; Savalli and Fox, 1999; Jones and Elgar, 2004; Smith and Blumstein, 2008).

Although our observations were carried out in laboratory, they were performed on a large group of individuals free to interact, thus mirroring the natural condition in which this species usually lives (e.g., Faleiro, 2006). Indeed, the cryptic habit of RPW (Giblin-Davis et al., 2013) makes behavioural interactions among individuals living inside the same palm impossible to observe.

Our study, despite the inherent limitation of laboratory studies (Campbell et al., 2009), represents a step forward compared to the previous reports focussed on single pair behaviour and highlights how the study of socio-sexual interactions, especially with a SNA ap-

proach may give interesting insight for the development and tuning of management techniques.

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SUPPLEMENTARY MATERIALS

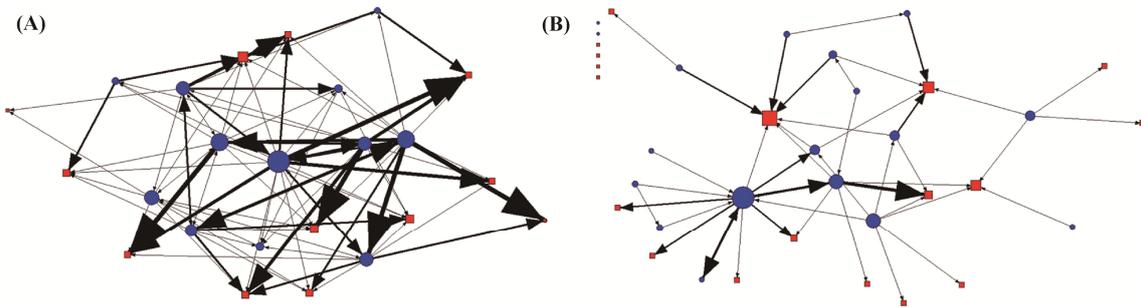


Fig. S1 Social networks of G2(A) and G3 (B), visualized with the Spring embedding technique

The three behaviours recorded were heterosexual mating, homosexual mating and forced mating interruption. Nodes represent individual weevils and edges represent interactions. Node size is proportional to degree (number of nodes with which the focal nodes had at least one interaction). Edges thickness is proportional to edge strength (number of interactions occurred, pooled for the three behaviours) and arrows point toward receivers. Unconnected circles represent individuals that never did nor received an interaction (isolates). Blue circles: males, red circles: females.

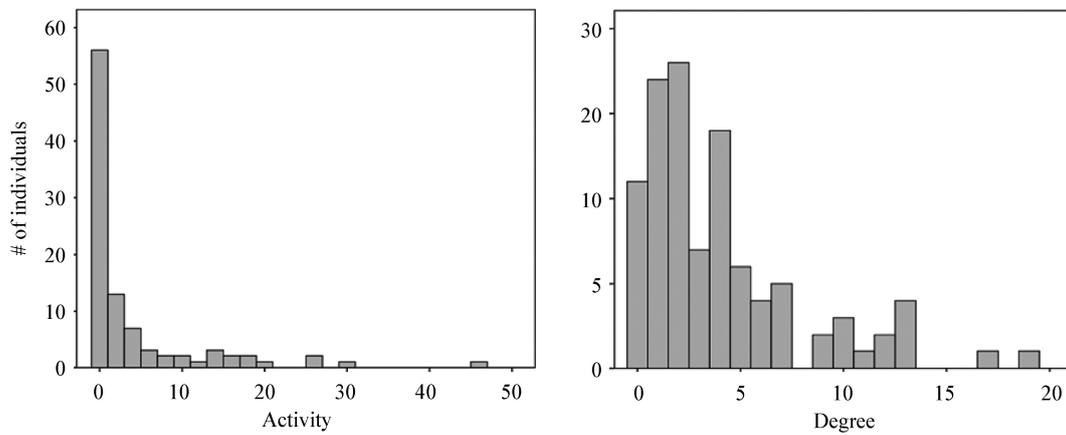


Fig. S2 Frequency distribution of activity and degree in the overall population

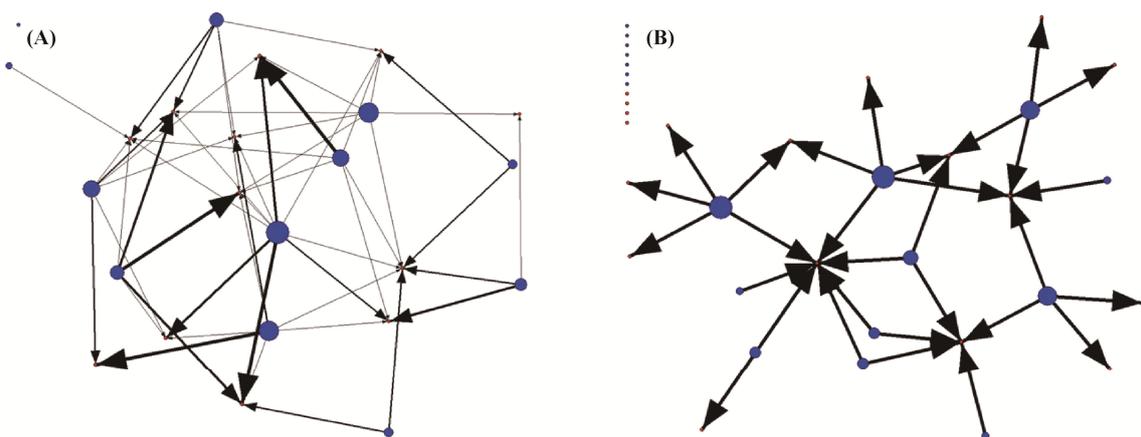


Fig. S3 Sexual interaction network of RPW G2 (A) and G3 (B) (considering heterosexual interactions only), visualized with the Spring embedding technique

Nodes represent individual weevils and edges represent interactions. Node size is proportional to the number of partners (number of nodes toward which the focal nodes performed at least one interaction). Edges thickness is proportional to edge strength (number of interactions occurred, only heterosexual mating were considered, which correspond to male reproductive effort) and arrows point toward receivers. Isolates are not reported. Blue circles: males, red circles: females.

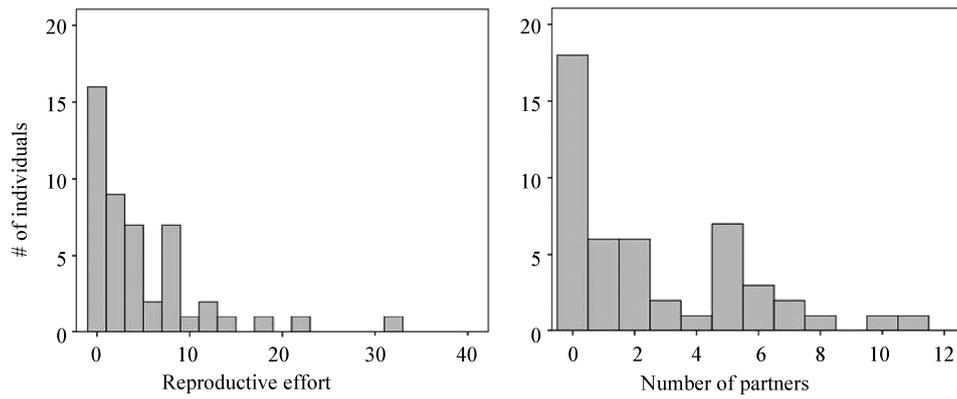


Fig. S4 Frequency distribution of male reproductive effort and number of partners in the overall population, considering only heterosexual matings

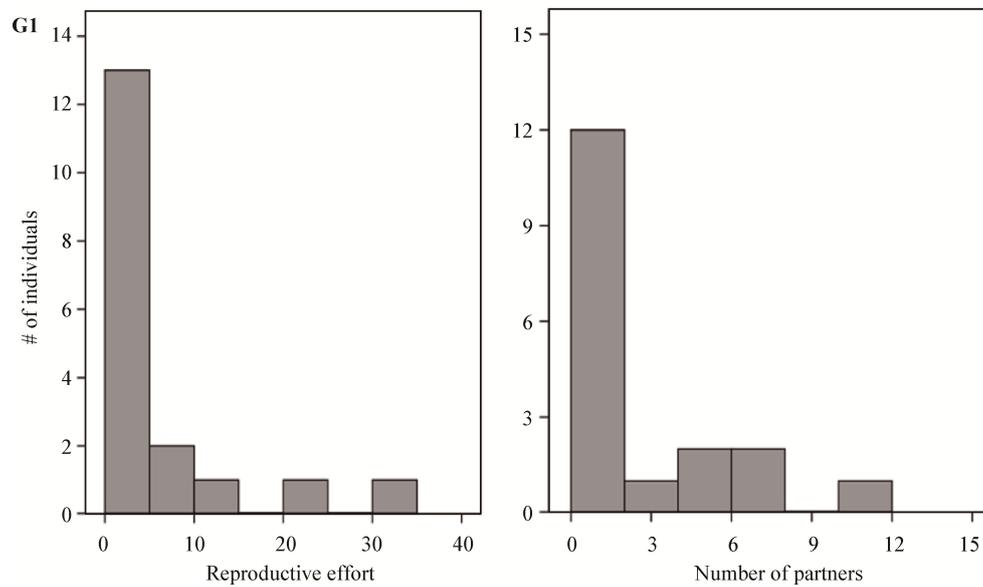


Fig. S5 Frequency distribution of male reproductive effort and number of partners in the G1 group, considering only heterosexual matings

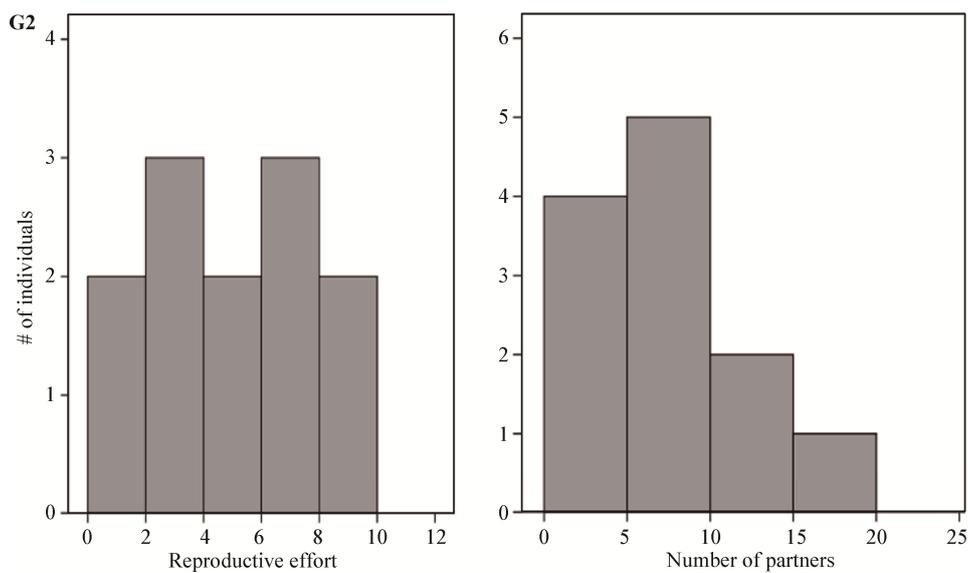


Fig. S6 Frequency distribution of male reproductive effort and number of partners in the G2 group, considering only heterosexual matings

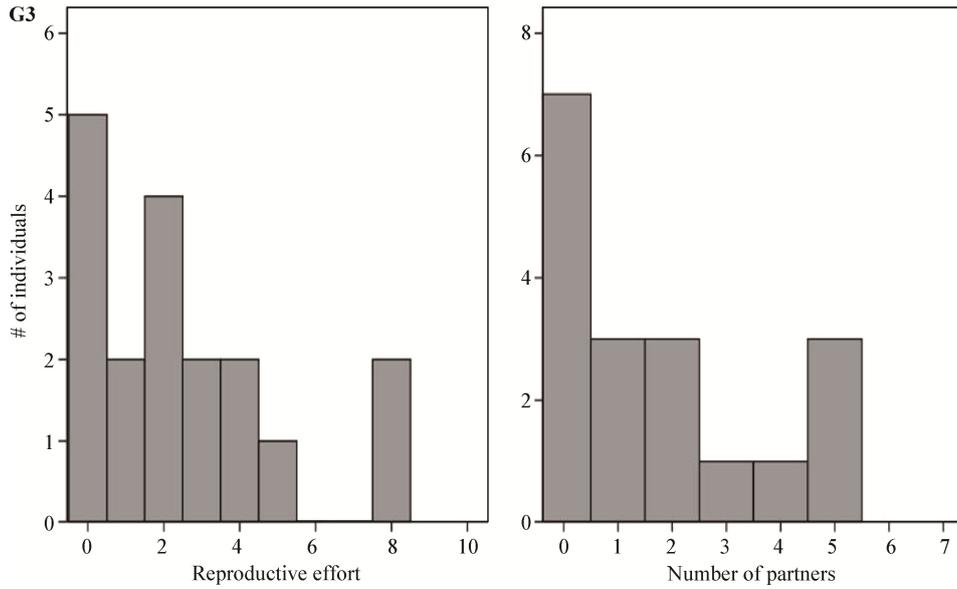


Fig. S7 Frequency distribution of male reproductive effort and number of partners in the G3 group, considering only heterosexual matings

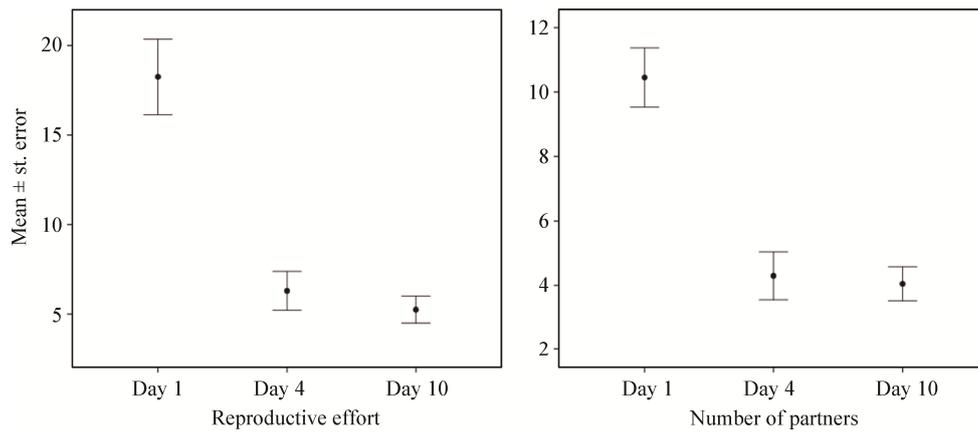


Fig. S8 Error bars report mean value and standard error of reproductive effort and number of partners for males of G4 at three different time points, day 1, day 4 and day 10