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1 Ecology and evolution of insect cognition

2

3 **Putting the ecology back into insect cognition**

4 **research**

5

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15

16

17 **Abstract**

18 Over the past decades, research on insect cognition has made considerable advances in
19 describing the ability of model species (in particular bees and fruit flies) to achieve cognitive
20 tasks once thought to be unique to vertebrates, and investigating how these may be
21 implemented in a miniature brain. While this lab-based research is critical to understand some
22 fundamental mechanisms of insect brains and cognition, taking a more integrative and
23 comparative view will help us making sense of this rich behavioural repertoire and its
24 evolution. Here we argue that there is a need to reconsider insect cognition into an ecological
25 context, in order to design experiments that address the cognitive challenges insects face in
26 nature, identify competing hypotheses about the cognitive abilities driving the observed
27 behavioural responses, and test them across different populations and species. Reconnecting
28 with the tradition of naturalistic observations, by testing animals in the field or in
29 ecologically-inspired setup and comparing the performances of individuals, is complementary
30 to mechanistic research in the lab, and will greatly improve our understanding of the role of
31 insect cognition, its the diversity, and the influence of ecological factors in its evolution.

32

33 Key words: insects, cognition, brain, behaviour, learning, memory, natural selection,
34 sociality, field-based research, cognitive ecology

35

36 “... *que se passe-t-il dans ce petit cerveau d’hyménoptère? Y a-t-il là des facultés soeurs des*
37 *nôtres, y a-t-il une pensée? Quel problème, si nous pouvions le résoudre; quel chapitre de*
38 *psychologie, si nous pouvions l’écrire!*” [... *what happens in this little brain of*
39 *Hymenoptera? Are there abilities similar to ours, is there a thought? What problem, if we*
40 *could solve it; what chapter of psychology, if we could write it!*]

41 Translated from Jean-Henri Fabre (Fabre, 1882, p405).

42

43 **1. Past and present of insect cognition research**

44 Famous naturalists such as Réaumur, De Geer, Latreille, Fabre, Darwin, Lubbock, to name
45 just a few, have played a considerable role in suggesting that insects, just like large-brained
46 animals, are capable of adapting to new situations through various forms of learning, memory
47 and information transfer. In the 20th century, the first ethologists made invaluable
48 contributions to our understanding of these processes, through experimental manipulations
49 and quantifications of insect behaviour in the field. Von Frisch (1915), for instance, used
50 artificial flowers to demonstrate colour discrimination by honey bees, before discovering the
51 symbolic communication by which foragers advertise the location of remote feeding sites to
52 their nestmates by displaying dances on the vertical honey combs (von Frisch, 1967).

53 Tinbergen manipulated the visual appearance of the nests of digger wasps with pine cones and
54 demonstrated that wasps use visual memories to orient themselves and return home
55 (Tinbergen, 1932).

56 Since then, generations of talented entomologists have described a rich diversity of
57 cognitive abilities by which insects sample, process and use information from their
58 environment to adapt their behaviour in different contexts (e.g. mate choice, foraging, egg
59 laying, navigation) at different levels (e.g. as individuals and as groups) and in a variety of
60 taxa (for recent reviews see: Collett et al., 2013; Feinerman and Korman, 2017; Giurfa, 2019,

61 2013; Papaj and Lewis, 2012; Perry et al., 2017). This research shows that model species
62 (especially bees and fruit flies) achieve ever more impressive cognitive tasks despite their
63 relatively simple neural system. At the individual level, bees are capable of learning concepts
64 (Giurfa et al., 2001), counting (Howard et al., 2018), optimising paths (Lihoreau et al.,
65 2012b), copying others (Alem et al., 2016), innovating (Loukola et al., 2017) and even
66 assessing their chances to solve a task (Perry and Barron, 2013). Some wasps can recognize
67 the faces of their nestmates (Sheehan and Tibbetts, 2016) and fruit flies can socially transmit
68 mate choice preferences across several generations, possibly leading to local traditions
69 (Danchin et al., 2018). At the collective level, ant and bee colonies often make faster and
70 more accurate decisions than isolated conspecifics when selecting food sources (Beckers et
71 al., 1990) or a nesting site (Sasaki et al., 2013; Seeley, 2010), and can efficiently solve mazes
72 (Goss et al., 1989) or transport large food items across complex environments (Gelbium et al.,
73 2015).

74 Together with the development of new technologies and methods in neurosciences
75 (Dubnau, 2014; Menzel, 2012), this research on insect cognition has progressively moved
76 from the description of sophisticated behaviour in the field to mechanistic investigations of
77 cognitive processes and their neural correlates in the lab. Significant progresses in
78 understanding insect brain organisation and function have been made using genetic mutants
79 (e.g. GAL4/UAS, optogenetics), various imaging techniques, drug injections or screening of
80 gene expression in targeted neuropiles (Guo et al., 2019). We now have a fairly good idea of
81 brain areas, neurons and molecular pathways involved in different forms of associative
82 learning in model species such as fruit flies, honey bees, some ants, moths, cockroaches and
83 crickets (Giurfa, 2013). In particular, the fruit fly *Drosophila melanogaster* has emerged as a
84 key genetic model to address these questions both because of the relative simplicity of its
85 nervous system (mapped at the level of synaptic connectivity (Zheng et al., 2018)) and its rich

86 behavioural repertoire (both individual and social levels (Sokolowski, 2010)), allowing for the
87 genetic dissection of sophisticated behaviours, such as place learning (Ofstad et al., 2011),
88 flight control (Dickinson and Mujires, 2016), courtship (Pavlou and Goodwin, 2013),
89 grooming (Hampel et al., 2015), memory-driven action selection (Owald and Waddell, 2015)
90 and collective movements (Ramdya et al., 2014).

91 Although very insightful, the fast development of lab-based mechanistic studies has
92 also reduced the scope of insect cognition research in several ways. First, the focus on the
93 molecular and genetic bases of cognitive processes has limited investigations to few model
94 species that may not express a cognitive repertoire representative of the estimated 5.5 millions
95 insect species (Stork, 2018). While it can be interesting to compare bees, ants and wasps
96 when considering social evolution within the social Hymenoptera (e.g. Farris, 2016;
97 Gronenberg and Riveros, 2009), the comparison with the more phylogenetically distant fruit
98 flies may be less informative (Brenowitz and Zakon, 2015). Second, studies on the
99 mechanisms of learning and memory often rely on hypotheses and paradigms inspired from
100 human experimental psychology that may sometimes bias interpretations of the results, and
101 limit the search for alternative (sometimes more parsimonious) explanations (e.g. Cheung,
102 2014; Guiraud et al., 2018). Third, research that is exclusively conducted in the lab presents
103 the risk of disconnecting subjects, behaviours and cognitive traits of interest from their natural
104 environment. Testing animals in very artificial setups in order to achieve a high level of
105 control on information available and behavioural responses, does not always allow for the
106 expression of the desired naturalistic behaviours (e.g. Niggebrügge et al., 2009). The
107 questions or approaches used to study insect cognition are often very different from situations
108 animals may face in nature (e.g. study aversive learning using electric shocks, conditioning
109 immobile harnessed insects, testing social insects in isolation). The animals themselves used
110 for testing cognitive abilities often come from long-term laboratory or commercial cultures in

111 which some traits may be inadvertently selected or counter selected (e.g. commercial
112 bumblebees, drosophila mutant strains). Fourth, the type and levels of stress animals are
113 exposed to may be highly different in the lab and in the field. This can be problematic since
114 several recent studies show that negative or positive experiences can induce emotion-like
115 states in insects that have consequences on their behaviour and performances in cognitive
116 tasks (e.g. drosophila: (Yang et al., 2013); honey bees: (Bateson et al., 2011); bumblebees:
117 (Perry et al., 2016)).

118 Here we argue that there is a need for complementing current lab-based insect
119 cognition research with more ecologically inspired studies in order to fully understand the
120 diversity and evolution of cognitive traits. In recent years, such approach known as “cognitive
121 ecology” has been fully embraced by behavioural ecologists and experimental psychologists
122 working on vertebrates and proved successful to advance knowledge on the ecological role
123 and evolution of bird and primate cognition (Dukas, 2008, 1998; Dukas and Ratcliffe, 2009;
124 Morand-Ferron et al., 2015; Pritchard et al., 2016). In what follows, we advance that time is
125 ripe for extending this approach to insects. First, we review conceptual frameworks that have
126 been proposed for the evolution insect brain and cognition. We then explain how taking into
127 account the ecological context in which cognitive traits are expressed in nature can help refine
128 these frameworks by designing field-inspired experiments, testing wild animals, bringing lab
129 controlled protocols to the field, as well as comparing more species. Finally, we discuss how
130 technological advances to study insect cognition in ecologically realistic conditions will help
131 develop this comparative approach, by dramatically increasing the number of cognitive tasks
132 and individuals that can be investigated.

133

134 **2. The evolution of insect brains and cognition**

135 While we are now getting a more accurate picture of what insects can and cannot do (Perry et
136 al., 2017), and which are the brain areas and neural circuits involved in some of these
137 operations (Giurfa, 2013), fundamental questions about why and how cognitive traits evolve
138 in these animals remain poorly understood.

139 Both social and ecological factors are expected to fashion the evolution of brains and
140 cognitive abilities (Shettleworth, 2009). Since early descriptions of the anatomy of insect
141 nervous system (Dujardin, 1850), many discussions about the evolution of insect brains and
142 cognitive abilities have focused on the influence of social factors (Strausfeld, 2012).
143 Following the ‘social brain hypothesis’ developed to explain the evolution of large brains in
144 social vertebrates, and in particular anthropoid primates (Byrne, 1996; Dunbar, 1998), two
145 hypotheses were recently proposed for insects. Gronenberg and Riveros (2009) suggested that
146 the transition from solitary to gregarious and colony-based social structures has required the
147 expansion of brain regions related to communication, large behavioural repertoires and
148 flexibility. However, behavioural specialization in socially advanced species with division of
149 labour may have led to reduced investment in brain regions underpinning a range of cognitive
150 operations not required anymore, thereby predicting a quadratic relationship between
151 increasing levels of social complexity and brain size (Gronenberg and Riveros, 2009).
152 O’Donnell et al. (2015) proposed that group communication relaxes the need for individual
153 information processing, resulting in a linear decrease of brain size (or brain size areas) with
154 increasing levels of sociality.

155 Despite many attempts to correlate brain sizes with metrics of social complexity in
156 different insect taxa, empirical supports for a social brain hypothesis are mixed (Farris, 2016;
157 Gordon et al., 2019; Kamhi et al., 2019, 2016; O’Donnell et al., 2015; Riveros et al., 2012).
158 Part of the problem may be methodological (e.g. coarse measures of social complexity and
159 brain sizes, lack of phylogenetical approaches), thus calling for broader comparative analyses

160 of neuro-anatomical and behavioural studies mapped on phylogenies (Godfrey and
161 Gronenberg, 2019; Lihoreau et al., 2012a). Another difficulty in testing these variants of the
162 social brain hypothesis lies in the unverified assumption that larger behavioural repertoires
163 require larger brains. In fact, many fundamental changes in the complexity of a nervous
164 system may not result in measurable volumetric differences and novel behaviour can emerge
165 from minimal rewiring of existing neurons (Chittka and Niven, 2009).

166 The strong focus on the importance of social factors for the evolution of brains and
167 cognitive capacities (especially in Hymenoptera) has somehow neglected a number of
168 alternative or complementary hypotheses that have been long developed by vertebrate
169 biologists, such as the importance of diet (DeCasien et al., 2017), maternal care (Curley and
170 Keverne, 2005) or spatial navigation (Jacobs et al., 1990). Ecological conditions are known to
171 fashion the evolution of insect sensory systems and brain anatomy (e.g. vision (Briscoe and
172 Chittka, 2001), olfaction (Hansson and Stensmyr, 2011)). However, the links between
173 ecological constraints and cognitive capacities have been little explored. In an attempt to test
174 these alternative hypotheses in Hymenoptera, Farris and Schulmester (2011) made a careful
175 evaluation of the architecture of the mushroom bodies (central brain structures involved in
176 various forms of visual, olfactory and bimodal memories (Strausfeld, 2012)) in a wide
177 diversity of species and mapped their lifestyles and neural structure onto an established
178 phylogeny. This analysis showed that relatively enlarged mushroom bodies, with elaborate
179 structure and visual and olfactory inputs, evolved 90 million years prior to sociality, in
180 solitary parasitoid wasps (Farris and Schulmeister, 2011). Presumably, the challenge of
181 acquiring spatial memories for locating preys and provisioning larvae (not sociality) may have
182 placed much higher cognitive demands in these first parasitoids than in their herbivorous
183 ancestors. In fact, this cognitive adaptation to spatial orientation may have later favoured the

184 evolution of central place foraging and the development of large societies sustained by highly
185 efficient visuo-spatial foragers (Farris, 2016).

186

187 **3. Towards a cognitive ecology of insects**

188 The emerging field of cognitive ecology provides a theoretical and methodological
189 framework to study the ecology and evolution of animal cognition (for reviews see (Dukas,
190 1998; Dukas and Ratcliffe, 2009)). This involves designing new hypotheses and experiments
191 based field observations, testing wild animals, bringing lab-controlled experimental protocols
192 in the field, taking into account the social context of the cognitive task, and comparing large
193 numbers of species with known ecologies and phylogenetic relationships. While this approach
194 has so far mainly been used for vertebrates, especially birds and mammals (Dukas, 2008;
195 Morand-Ferron et al., 2015; Pritchard et al., 2016), below we highlight some key recent
196 examples in insects.

197

198 Identifying new questions and hypotheses from field observations

199 Field observations are necessary to identify the types of problems animals must solve in their
200 everyday life and how they might do so. The natural environment often contains much more
201 relevant cues for the animals than typically assumed which structures the kind of information
202 they can acquire. Observing insects in their natural environment is thus a fundamental step to
203 design questions, identify competing hypotheses, develop experimental protocols and
204 potentially change paradigms, be the research later conducted in the field or in the lab.

205 Field observations are particularly important in insect navigation research since spatial
206 orientation behaviours are not always easily expressed in the lab, because of the limited
207 spatial scales of lab-setups and the incomplete set of environmental cues available to insects.

208 In bees, field observations have recently moved the historical focus on single destination

209 route following to multi-destination route learning and optimisation (Lihoreau et al., 2013). In
210 an attempt to study long-distance pollination by orchid bees in the Costa Rican rain forest,
211 Janzen (1971) observed that some individuals often visited the same set of plants each day,
212 probably in the same order. Given that bees are often assumed to visit hundreds of flowers
213 during a single foraging trip (von Frisch, 1967), this anecdotic observation has initiated
214 several research programs investigating how bees develop routes linking many familiar sites
215 (Lihoreau et al., 2012b; Ohashi et al., 2007; Woodgate et al., 2017), for how long route
216 memory is effective (Thomson, 1996), and how individuals achieve this behaviour while
217 minimizing competition with other nectar foragers (Ohashi et al., 2008; Pasquaretta et al.,
218 2019). In ants, field observations have raised new questions about how insects use
219 environmental cues to solve orientation challenges. In the Australian desert, thermal
220 turbulences due to solar heating of the ground create frequent wind gusts and it is not rare to
221 see ants getting blown away from their familiar route. Even a small displacement of a few
222 meters (i.e. several hundreds of body lengths for an ant) constitutes a big challenge for the ant
223 to find back its original position. Based on this observation, desert ants (*Melophorus bagoti*)
224 were observed reo-orientating in the field after being experimentally displaced by wind gusts
225 of leaf blower into a dark pit (Wystrach and Schwartz, 2013). When released at windless
226 unfamiliar locations, ants headed in a compass direction opposite to the one they had been
227 blown away, thus functionally increasing their chance of returning to familiar areas. Analyses
228 of ant behaviour indicate that encoding of wind direction relative to sun position occurs
229 before being displaced, while clutching the ground to resist the wind (Wystrach and Schwartz,
230 2013). Field observations that ball-rolling dung beetles (*Scarabaeus lamarcki*) also appear to
231 use wind in addition to the sun for spatial orientation have raised the question of how insects
232 may use multimodal compass cues for navigation and inspired lab experiments in which sun
233 and wind cues can be delivered in a tightly controlled manner (Dacke et al., 2019). In this

234 setup, beetles were found to register information provided by the sun and the wind, and
235 directional information can be transferred between these two sensory modalities, suggesting
236 that they combine in the spatial memory network in the beetle's brain. This flexible use of
237 compass cue preferences relative to the prevailing visual and mechanisms scenery provides a
238 simple, yet effective, mechanism for enabling compass orientation at any time of the day
239 when one type of cues may not be available (Dacke et al., 2019).

240 Field observations can also been pivotal to understand cognitive processes in
241 populations of animals, such as the collective decision-making processes underpinning the
242 onset of insect swarms (Bazazi et al., 2008; Simpson et al., 2006). During population
243 outbreaks, Mormon crickets (*Anabrus simplex*) form marching bands of several kilometres
244 long, comprising millions of individuals moving en masse (Sword et al., 2005). Field
245 observations of migratory bands indicated that many dead insects were left behind, as well as
246 some carcasses of small vertebrates, suggesting that populations of sedentary herbivorous
247 crickets swarm in response a local depletion of key nutrients (Simpson et al., 2006). Giving
248 migrating crickets a choice between artificial diets varying in their nutritional composition in
249 the field demonstrated that crickets in migratory bands are deprived of protein and mineral
250 salts, which triggers their cannibalistic interactions. The crickets are in effect on a forced
251 march, whereby individuals move ahead to try to eat conspecifics while escaping cannibalism
252 by others in their back (Simpson et al., 2006).

253

254 Testing wild animals

255 Running experiments on wild animals offers the opportunity to assess important inter-
256 individual variations in behaviour and cognition that are potentially shaped by environmental
257 conditions, thereby providing a link between cognitive performances and the ecological
258 context (Morand-Ferron et al., 2015).

259 In fruit flies (*D. melanogaster*), the utilization of wild-caught individuals for
260 behavioural experiments has revealed the existence of natural allelic variations of the gene
261 *foraging*, which encodes a cGMP-dependant protein kinase (PKG) that affects the motor
262 behaviour and social interactions of larvae and adults (Sokolowski, 1980). Sitter flies (*for*^S)
263 are more sedentary and tend to aggregate within food patches, whereas rover flies (*for*^R) to
264 move more within and between food patches and are less gregarious (Sokolowski, 2010).
265 These two natural behavioural variants are maintained at appreciable frequencies (ca. 70%
266 rovers, 30% sitters) in nature (Sokolowski, 1980) and in the lab through negative frequency
267 dependent selection (Fitzpatrick et al., 2007). Rovers and sitters also show important
268 differences in their cognitive abilities. Rovers express stronger proboscis extension responses
269 following a sucrose stimulation of their tarsi and show slower habituation of this response
270 after multiple stimulations than sitters (Scheiner et al., 2004). Rovers develop better short-
271 term aversive olfactory memory but poorer long-term memory than sitters (Mery et al., 2007).
272 Interestingly, these two behavioural variants also differ in their ability to use social
273 information. In a spatial task, where flies must learn to locate a safe zone in an aversively
274 heated arena (i.e. invertebrate version of the Morris water maze), rovers rely more on personal
275 information whereas sitters tend to primarily use social cues (Foucaud et al., 2013). These
276 results suggest that both the utilization of information types and the cognitive performances of
277 the two genotypes are co-adapted with their effects on foraging behaviour: the highly
278 exploratory rovers could particularly benefit from fast learning based on individual
279 information, whereas the more sedentary sitters should benefit more from social information
280 and good long-term memory.

281 Wild populations are characterised by natural levels of genetic diversity that can
282 greatly impact levels of behavioural variability in cognitive tests. Experiments with German
283 cockroaches (*B. germanica*) from different laboratory strains showed that individuals can

284 discriminate between conspecifics with different genetic backgrounds, favouring aggregations
285 with partners from the same strain (Rivault et al., 1998; Rivault and Cloarec, 1998) but
286 mating with partners from different strains (Lihoreau et al., 2007). Intra-strain (kin)
287 discrimination, however, was only demonstrated later, in studies using wild-caught
288 cockroaches sampled in separate geographic areas, showing that behavioural discrimination is
289 based on quantitative differences in chemical signatures (i.e. cuticular hydrocarbon profiles)
290 correlated with the genetic distance between individuals (Lihoreau et al., 2016b). The
291 potential lack of genetic diversity in lab cultures maintained for long periods of time (highly
292 inbred, no information about genetic background) may be a reason why kin recognition has
293 been observed so rarely in insects (Fellowes, 1998; van Zweden and D’Ettorre, 2010).

294

295 Bringing experimental protocols in the field

296 Insect cognition research is largely based on well-defined paradigms designed to investigate
297 specific cognitive traits (Giurfa, 2013). While this provides the advantage of allowing the
298 identification of what animals can do, it may not, however, always reflect what animals
299 actually do in the wild (Pritchard et al., 2016).

300 Firstly, important stimuli yielding information necessary for the expression of targeted
301 behaviour may be absent in the lab. This is well illustrated by studies on visual cognition.
302 Bees are capable of various forms of visual associative learning and memories used to locate
303 and discriminate flowers, as well as developing routes between them (Avarguès-Weber et al.,
304 2011). To control for the visual experience of bees, the spatial distribution of flowers and
305 their rewarding value, bees spatial foraging strategies have been studied in the lab using
306 artificial flowers in small flight arenas, flight rooms or greenhouses. In many bee species,
307 foragers allowed to exploit an arrays of artificial flowers over several consecutive hours tend
308 to develop repeatable flower visitation sequences (Lihoreau et al., 2010; Ohashi et al., 2007;

309 Saleh and Chittka, 2007), a behaviour called “trapline foraging” (Thomson et al., 1997).
310 Replicating these experiments in the field, using a harmonic radar to record the flight
311 trajectories of individual bees at much larger ecologically relevant spatial scales, revealed that
312 bees establish routes minimizing travel distances between all flowers and the nest based on
313 long-term memories (Lihoreau et al., 2012b; Woodgate et al., 2017). In this case, both the
314 increased spatial scales (e.g. longer travel distances associated to higher energetic costs) and
315 the access to celestial cues (e.g. sun compass) dramatically accelerated the dynamics of route
316 formation and improved the optimization performance of bees in the field setup.

317 Another major advantage of adapting lab experiments to the field is to avoid potential
318 sources of stress inherent to the lab. Even if insect species can be brought into the lab and the
319 spatial scale and the information available to the insects were appropriate for understanding
320 the behaviour of interest, the insect itself may still experience the lab task very differently
321 than if it were presented with an analogous task in the wild. Again, research on bee visual
322 cognition provides a good illustration of how lab-based protocols can be adapted to the field
323 to tackle this problem. One of the most common paradigm for investigating learning and
324 memory in honey bees is the conditioning of the proboscis extension reflex (PER), which
325 tests for associations between an unconditional stimulus (sucrose reward) and a conditional
326 stimulus (e.g. colour or scent) in harnessed bees (Takeda, 1961). This approach has the
327 advantage of enabling the control for the timing of stimulus presentation (e.g. sequence of
328 stimulus exposure, number of trials, inter-trial duration). While PER conditioning has been
329 incredibly insightful to study olfactory cognition at the behavioural, neurobiological and
330 molecular levels (Giurfa and Sandoz, 2012), it has always given poor or contrasted results
331 with visual stimulations (e.g. some authors report the necessity to amputate antennae to obtain
332 good learning (Kuwabara, 1957; Niggebrügge et al., 2009)) and have never reached the usual
333 levels observed in free-flying bees (Avarguès-Weber et al., 2011). Considering that bees

334 predominantly use vision in flight, motion cues probably provide more natural visual context
335 that participate to maintain a close dependence between visual and motor processing. The
336 immobilization of the bee in visual-PER studies undoubtedly disrupts this feedback loop
337 (Avarguès-Weber and Mota, 2016). To address this issue, Muth et al. (2018) developed a
338 field version of PER conditioning with freely moving insects in which animals reach high
339 performance levels. This new protocol allows for testing visual associative learning and
340 memory of different species of bees in a less stressful environment, while controlling for
341 stimulus presentation as well as allowing tests in field conditions on wild populations (Muth
342 et al., 2018).

343

344 Taking into account the social context

345 The difference between the lab and the natural conditions under which an animal usually
346 learns is sometimes not just physical (Pritchard et al., 2016). While many standard cognitive
347 tests are performed on isolated insects (Giurfa, 2013; Menzel, 2012), key model species such
348 as drosophila, honey bees and ants live in groups (Sokolowski, 2010; Wilson, 1971). A
349 number of social factors may thus influence what the insects can learn or how they express
350 their learning behaviour.

351 At the most basic level, some behaviours are simply not expressed out of the social
352 context. In an attempt to test the hypothesis that division of labour in social insects emerges
353 from inherent inter-individual variation in response thresholds to environmental stimuli (i.e.
354 the response threshold hypothesis (Beshers and Fewell, 2001)), the behaviour of individual
355 ants (*Temnothorax rugatulus*) was compared in different social contexts. When isolated, ants
356 show highly variable responses to task-associated stimuli and these responses are not
357 correlated to their behaviour in the colony, suggesting that testing ants outside of a social
358 context alters the meaning or salience of the experimental stimuli and thus the observed

359 behavioural response (Leitner et al., 2019). These social effects on cognition can also be
360 developmental. In many gregarious insects, prolonged periods of social isolation can have
361 dramatic developmental consequences and induce long-term behavioural disturbances known
362 as “group effects” (Grassé, 1946). In the German cockroach (*Blattella germanica*),
363 individuals experimentally reared in isolation during nymphal development show lower
364 exploratory activities, foraging behaviour, and abilities to process social stimuli as adults
365 (Lihoreau et al., 2009). This behavioural syndrome of social isolation can be partially rescued
366 through social contacts artificially provided to cockroaches through mechanical stimulations
367 (Lihoreau and Rivault, 2008; Uzsak and Schal, 2013).

368 Social interactions can also modulate learning and memory performances. In fruit flies
369 (*D. melanogaster*), social interactions facilitates the retrieval of olfactory memory (Chabaud
370 et al., 2009). Flies trained to associate an electric shock to an odour in a T-maze develop two
371 forms of long-lasting memories depending on inter trial intervals: long-term memory (LTM)
372 is formed after spaced conditioning (short intervals), whereas anaesthesia-resistant memory
373 (ARM) is formed after massed conditioning (long intervals) (Margulies et al., 2006).
374 However, flies have higher ARM scores when tested in groups than in isolation (Chabaud et
375 al., 2009). This social effect is independent of the social condition of training, of the
376 experience of other flies in the group and is specific to ARM, indicating that it does not
377 simply result from aggregation dynamics. Presumably, trained flies produce stress signals
378 (e.g. CO₂ (Yang et al., 2013)) that alarms their conspecifics and enhances their attention or
379 motivation to respond during memory retrieval. In honey bees (*A. mellifera*), social condition
380 during breeding influences olfactory learning. Adults raised in large groups show better
381 learning but no higher memory scores than conspecifics raised in small groups or in complete
382 isolation (Tsvetkov et al., 2019). These differences are correlated with changes in dopamine

383 levels in the brain suggesting that social interactions modulate learning through the biogenic
384 amines.

385 Being in a group can also dramatically improve the speed and accuracy of decision-
386 making through collective acquisition and processing of information, a phenomena known as
387 “swarm intelligence” (Couzin, 2009; Feinerman and Korman, 2017; Seeley, 2010). In house
388 hunting ants (*T. rugatulus*), collective decisions for the selection of a new nest site emerge
389 from a competition between recruitment efforts by different individuals in the form of tandem
390 running (i.e. an experienced ant drags a naïve ant towards a site) at different sites (Franks et
391 al., 2002). When given a choice between potential nest sites varying in quality (e.g. light
392 intensity), ant colonies can effectively compare a larger option set than individuals (Sasaki
393 and Pratt, 2012) and are less vulnerable to irrational preference shifts induced by decoys
394 (Sasaki and Pratt, 2011). However, this social advantage varies with the difficulty of the task
395 to solve (Sasaki et al., 2013). For a difficult choice (i.e. small differences of light intensity
396 between nests), solitary ants have a relatively high probability of accepting the worst nest,
397 because they rely on quality dependent acceptance probabilities that differ little for similar
398 nests. Colonies do much better because the colony’s choice emerges from a competition
399 between recruitment efforts accentuated by a positive feedback loop and a quorum rule
400 (Sasaki et al., 2013). For an easy choice (i.e. large differences in light intensity between
401 nests), acceptance probabilities diverge rapidly with comparison, allowing solitary ants to
402 make the right choice with high probability. Thus in this case social information only adds
403 little benefit to colonies if not a cost (when random fluctuations lead the colony towards the
404 wrong choice).

405

406 Comparing species

407 Rigorous comparisons of the cognitive performances of individuals of the same species or
408 different species that are either closely or distantly related can greatly enhance our
409 understanding of how cognition is shaped by natural selection (Godfrey and Gronenberg,
410 2019).

411 Studies of closely related species with known ecologies is a powerful means to tease
412 apart selective forces that drive the evolution of specific cognitive traits. In paper wasps such
413 comparison demonstrates the importance of sociality in the evolution of visual cognition
414 (Sheehan and Tibbetts, 2011). Queens of *Polistes fuscatus* cooperate to found, defend and
415 provision their colony. These wasps live in strict hierarchical societies in which individuals
416 recognise every other colony members based on long-term memories of facial masks
417 (Sheehan and Tibbetts, 2008). By contrast, queens of *P. metricus* found colonies alone and do
418 not require face recognition. When presented images of normal wasp faces, manipulated wasp
419 faces, simple geometric patterns or caterpillars (i.e. the typical prey of these wasps) in an
420 aversive conditioning paradigm in a Y-maze, *P. fuscatus* wasps learn to recognize correctly
421 configured wasp faces more quickly and more accurately than they did with other images,
422 indicating that face learning is specific to faces in this species (Sheehan and Tibbetts, 2011).
423 *P. metricus* wasps, however, perform better in pattern and caterpillar discrimination. In terms
424 of gross neuroanatomy, there are no discernible differences between the visual system of *P.*
425 *fuscatus* and closely related species that do not show face recognition (Gronenberg et al.,
426 2008). It is therefore likely that the neural circuitry used by insects for prey recognition has
427 been co-opted for face recognition, provided minor adjustments. In parasitoid wasps that lay
428 eggs in animal hosts, differences in the spatial distribution of preys seems to determine major
429 differences in olfactory memory dynamics (Smid et al., 2007). *Cotesia glomerata* and *C.*
430 *rubecula* wasps coexist in the same environments and lay their eggs in caterpillars. These
431 parasitoids are known to learn to associate plant odours with the presence of caterpillars

432 during an oviposition experience on a plant (Lewis and Takasu, 1990). When wasps of both
433 species are trained to oviposit on caterpillars on a neutral host plant and then given a choice
434 between the neutral host plant and their preferred host plant (cabbage), *C. glomerata* show
435 memory formation after fewer trials and faster memory consolidation than *C. rubecula* (Smid
436 et al., 2007). This difference in memory dynamics reflects the difference in foraging ecology
437 of the two species: *C. glomerata* exploits gregarious hosts and may benefit to learn from one
438 massed experience on a single encounter with a plant, whereas *C. rubecula* exploit solitary
439 hosts and may use more experiences and more time to evaluate information from many
440 different plants before long-term memory is formed.

441 Comparing distantly related species can help identify cognitive traits that are
442 conserved or are convergent across insect lineages. In recent years, the finding that many
443 insect taxa are capable of social learning, suggests that this cognitive ability once thought to
444 be unique to vertebrates has evolved several times in insects. Forms of social learning have
445 been demonstrated in insects exhibiting various levels of social organisation, including social
446 bees that can learn new flower preferences (Worden and Papaj, 2005) or foraging techniques
447 (Alem et al., 2016; Loukola et al., 2017), gregarious fruit flies that can learn preferences for
448 oviposition sites (Battesti et al., 2015) or mating partners (Danchin et al., 2018), or even
449 solitary field crickets that learn about the presence of danger (Coolen et al., 2005). This
450 comparative research demonstrates that insect social learning is not a specific adaptation to
451 social life but may rather involves fundamental associative learning processes common to
452 many species and used in an asocial context (Leadbeater and Dawson, 2017).

453

454 **4. Future directions**

455 Perhaps with the exception of navigation research (Collett et al., 2013), ecologically-inspired
456 studies of insect cognition are still relatively scarce, presumably because of the technical

457 difficulties to run controlled experiments with many insect species in their natural environment
458 (e.g. fast moving animals, large spatial scales, large numbers of individuals etc.). However,
459 several technological advances to quantify cognitive performances on freely moving insects in
460 the field or in field-realistic virtual environments in the lab hold considerable promises for the
461 development of insect cognitive ecology combining field and lab approaches.

462

463 Automated quantification of cognitive performances

464 A major limitation of current insect cognition research is that many experiments involve long
465 protocols (e.g. training sessions over several days (Perry et al., 2016)) with relatively low
466 levels of success (e.g. low learning scores (Avarguès-Weber and Mota, 2016)), often resulting
467 in small sample sizes that do not enable for analyses of cognitive variability. Developing a
468 truly comparative analysis of cognitive performances within individuals through time, as well
469 as between individuals, population and species requires the development of non-invasive
470 automated systems to record behavioural data on large numbers of insects over long periods
471 of times.

472 This can be achieved by automatizing cognitive protocols. Although many standard
473 protocols have been improved for automatically controlling the presentation of conditioned
474 and unconditioned stimuli to animals (e.g. appetitive olfactory conditioning in bees (Giurfa
475 and Sandoz, 2012), aversive visual conditioning in bees (Kirkerud et al., 2013), aversive
476 olfactory conditioning in drosophila (Jiang et al., 2016)), the full automation of experimental
477 setups for conducting cognitive tests is still rare. A recent successful example includes the
478 development of arrays of automated feeders fitted with tracking systems to test flower
479 choices, spatial learning and social interactions in freely flying bees in the lab (Ohashi et al.,
480 2010) and in the field (Lihoreau et al., 2016a). In this approach, a large number of insects can

481 self-train for several consecutive days without the intervention of an experimenter
482 (Pasquaretta et al., 2019).

483 Advances in automated movement tracking systems now also enable to quantify the
484 behaviour of individual insects, while walking or flying, at various spatial and temporal
485 scales, in the lab and in the field. These include computer vision (e.g. Pérez-Escudero et al.,
486 2014), radio frequency identification (e.g. Stroeymeyt et al., 2018), telemetry (e.g. Kissling et
487 al., 2014), and radar tracking (e.g. Riley et al., 1996). Recent studies have begun to
488 complement these behavioural measures with continuous recording of fitness data, population
489 dynamics and environmental conditions (e.g. Crall et al., 2018). In bee research, for instance,
490 connected hives (i.e. bee hives equipped with sets of sensors) can be used for the continuous
491 monitoring of colony traits (e.g. temperature, humidity, weight, sound, traffic of foragers,
492 social interactions, nectar and pollen collection) and environmental conditions (e.g. weather,
493 air pollution) (Bromenshenk et al., 2015). This technological advance has opened the door for
494 a real-time assessment of the link between insect cognitive performance, in-nest behaviour,
495 colony health status, environmental quality and stress exposure (Meikle and Holst, 2015).

496 High-throughput monitoring of insect behaviour can only be insightful if combined with
497 modern statistical methods to automatically analyse behavioural data. Approaches of machine
498 learning and statistical physics are increasingly used to run unsupervised behavioural
499 classification enabling to handle large behavioural datasets, discover features that humans
500 cannot, and develop standard metrics for comparing data across species and labs with only few
501 prior assumptions (Brown and de Bivort, 2018; Egnor and Branson, 2016).

502

503 Virtual reality on freely moving insects

504 The development of ecologically inspired lab-based experiments in which animals can
505 express naturalistic behaviours under tightly controlled conditions is complementary to field

506 research. While many classical protocols for testing learning and memory in the lab requires
507 to immobilize insects (e.g. (Giurfa and Sandoz, 2012)), recent progresses in virtual reality
508 techniques now provide unprecedented opportunities to test freely behaving animals in
509 complex (ecologically relevant) virtual environments, in which cues can be manipulated
510 independently, in ways that would be impossible to achieve in traditional experiments
511 (Stowers et al., 2017). These new systems in which the natural sensorimotor experience of
512 animals is conserved, facilitate detailed investigations into neural function and behaviour.
513 Virtual reality for freely moving animals has recently been used to elicit naturalistic object
514 responses (e.g. make objects appear, disappear, or even be at apparent distances) in freely
515 walking and flying insects. For instance, flying bumblebees (*Bombus terrestris*) can be
516 trained to search for virtual feeding platform or avoid virtual obstacles displayed on a screen
517 on the ground of a flight arena (Frasnelli et al., 2018).

518 Future developments of technologies to measure neural activities in freely moving
519 insects will considerably advance investigations of brain function underpinning these
520 naturalistic behaviours (Marescotti et al., 2018). Combining these technologies to virtual
521 reality will allow researchers to study the mechanistic basis of behaviour under conditions in
522 which the brain evolved to operate, thereby facilitating the dialogue between field and lab
523 cognitive experiments in ecologically relevant conditions.

524

525 **5. Concluding remarks**

526 In the 1980s and the 1990s, the intersection of behavioural ecology and experimental
527 psychology led to the new field of cognitive ecology (Dukas, 1998; Dukas and Ratcliffe,
528 2009) as researchers began to base their hypotheses on the natural history of different species
529 to test predictions about the cognitive abilities of these animals. This approach has been taken
530 with success by researchers working on large-brained animals (Morand-Ferron et al., 2015),

531 but is still little embraced by entomologists. Here we argue that there is a need for developing
532 an ecologically inspired research on insect cognition to develop a comprehensive
533 understanding of both its mechanisms and evolution.

534 Beyond behavioural ecologists, such approach will benefit to the broad community of
535 researchers interested in insect cognition. Considering the ecological context of cognition will
536 likely help ethologists to make sense of the rich cognitive repertoire of insects observed in the
537 lab (e.g. What does it mean for an insect colony to have optimistic and pessimistic foragers?
538 Why should insects count?) and perhaps refine mechanistic explanations by asking alternative
539 hypotheses inspired from field observations. Ecological considerations of cognition may also
540 help neurobiologists and experimental psychologists interested in the evolution of cognition
541 to understand the role of environmental factors in shaping animal behaviour and cognitive
542 abilities. As the cognitive abilities of more species are studied in the environment in which
543 such processes evolved, the prospects of a truly comparative study of cognition look bright.
544 Importantly, the ecologically-inspired approach is complementary with lab-based mechanistic
545 explorations. Some of these explorations can also be performed in the field, for instance using
546 selective drugs (Sovik et al., 2016) or inhibitor of gene expression (Cheng et al., 2015) to
547 identify physiological pathways underpinning cognitive operations in conditions where
548 animals may be in better position to fully express their cognitive repertoire.

549 Ultimately the dialogue between ecologically-based and lab-based approaches will
550 help develop a more integrative understanding of insect cognition with the potential to
551 illuminate broader scale ecological phenomena. For instance, detailed studies of the sublethal
552 effects of pesticides on bee learning and memory (Stanley et al., 2015) combined with field
553 monitoring of population dynamics (e.g. Henry et al., 2012) have provided a robust
554 explanation for colony collapse and the broader declines of pollinator populations (Klein et
555 al., 2017). Growing evidence show that cognitive processes observed in individual organisms

556 result from complex interactions between components at different levels of organisation (gut
557 microbiota, group, parasites and pathogens, environmental stressors) (Couzin, 2009; Cryan
558 and Dinan, 2012). Considering these ecological interactions and their consequences
559 throughout levels of organisations is a major challenge for insect cognition research in the
560 decades to come.

561

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568

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