

Context and control: behavioural ecology experiments in the laboratory

Dana L. M. Campbell^{1,*,#}, Susan A. Weiner^{2,#}, Philip T. Starks² & Mark E. Hauber^{1,3}

¹ School of Biological Sciences, University of Auckland, PB 92019, Auckland, 1142, New Zealand
(*corresponding author's e-mail: dcam047@aucklanduni.ac.nz)

² Biology Department, Tufts University, Medford MA 02155, USA

³ Department of Psychology, Hunter College, New York, NY 10065, USA

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Behavioural ecology is the study of the ecological and evolutionary bases for variation in animal behaviour, answering proximate and ultimate questions of why animals behave the way they do. The laboratory setting enables the isolation and control of specific variables, the removal or randomisation of confounding factors and simplifies the tracking of an individual's behaviour. Laboratory experiments, in parallel and in comparison with field studies, are valuable for answering specific questions and certainly most ecological investigations can benefit from a combined experimental approach. Here we focus on four model areas of behavioural ecological research: mate selection, nepotism, foraging and dominance. Using both vertebrate and invertebrate examples we consider the advantages and disadvantages of laboratory experiments and the unique information they can provide, including a comparison of three laboratory research contexts; neutral, natural and contrived. We conclude by describing how laboratory studies can help us to understand the contexts in which behavioural variation occurs in the natural environment.

Introduction

Behavioural ecology is a popular and active field of research that employs integrative approaches to study both the ecological and evolutionary bases for animal behaviour. Behavioural ecology endeavours to determine the role of behaviour in enabling individuals' adaptations and constraints to the surrounding environment (Krebs & Davies 1997). The initial research and observations of behaviourists (ethologists and comparative psychologists) demonstrated that though highly vari-

able, certain behavioural patterns can also be exceptionally characteristic and descriptive at the level of each species (Lorenz 1937, Tinbergen 1963, von Frisch 1974). Together with the development of sophisticated theory and extensive experimentation this has led to the growth of the thriving field of behavioural ecology, which connects individual and phylogenetic variation in behaviours with ecological variables and evolutionary histories (Krebs & Davies 1997).

In behavioural ecology experimentation, researchers aim to answer four focal types of

These authors contributed equally to this work.

questions, as famously coined by Tinbergen (1963), and divide research areas into two major fields: proximate and ultimate causes of behaviour. Proximate questions look directly at the *causal* and *ontogenetic* factors that influence observed behaviours, whereas ultimate questions, in turn, are concerned with the *functional* adaptations and *evolutionary* processes that may have imparted a selective advantage to a certain behavioural trait (Tinbergen 1963, Krebs & Davies 1997, Drickamer *et al.* 2002). These questions are essential focal points for behavioural ecologists seeking a complete understanding of any and all behaviours observed in nature and examined in the laboratory.

Field research on animals in their natural environment can provide the most appropriate and richest source of information on both the relationships of organisms to individual environmental variables and the relationships between multiple organisms within specific environmental parameters (Simberloff 2004, McGill *et al.* 2006, Johnson & Stinchcomb 2007, Underwood 2009). However, it is clear that in the field setting there are also countless external variables beyond the realm of experimental control that may be influencing the particular target behaviours we wish to study. It is in this regard that we take organisms into a laboratory setting to facilitate more extensive control over external environmental and, potentially, internal physiological and motivational variables, as well as limiting the number of interacting species or individuals that can all impact on behavioural outcomes. Of course a captive approach does limit the interpretations and applicability of results when the target species is isolated from influencing factors that may be present in a natural field situation (Table 1). Therefore, the chosen research setting of field vs. laboratory must ultimately depend on the question to be answered (Ford 2009), although undoubtedly many research topics in animal behaviour can benefit from a combination of both laboratory and field techniques. Accordingly, when we surveyed the literature of experimental techniques used in all published articles over 12 months of both *Animal Behaviour* and *Behavioral Ecology* (July 2006–July 2007) we found that most of studies that occurred solely in the laboratory (including experiments on captive

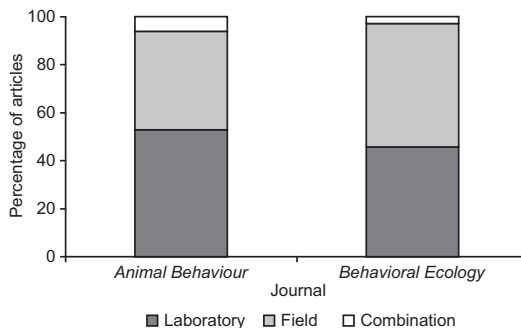


Fig. 1. The percentage of surveyed experimental articles in *Animal Behaviour* ($n = 246$) and *Behavioral Ecology* ($n = 208$) that were carried out in the 'Laboratory', 'Field' or a 'Combination' of Laboratory and Field settings. Although similar in overall proportions, *Behavioral Ecology* published more field only and fewer laboratory and combination studies ($\chi^2 = 6.34$, $df = 2$, $p = 0.042$).

populations in reasonably natural enclosures) or solely in the field (research on wild animals which may or may not have been handled by investigators to individually mark/tag for identification) and very few studies that looked at the focal animals' behaviour in both settings (Fig. 1).

Here we will focus on discussing varying settings and methods used solely in the laboratory under the realm of several major types of behavioural ecological sub-disciplines that animal behaviourists are actively engaged in investigating. We present both vertebrate and invertebrate exemplars and discuss what these methods uncover and what possible limitations the laboratory environment places on interpretation. We also discuss three varying laboratory research contexts that may be employed, outlining their main advantages and disadvantages. The methods and topics presented here are not in any way exhaustive, rather they highlight the range of techniques currently available in a laboratory setting for application in behavioural ecology research.

Four sample questions in behavioural ecology research

Within the field of behavioural ecology, researchers can set out to answer questions within several major areas of animal behaviour that can be explored in the laboratory setting. We selected studies that fall into the four categories of mate

selection, nepotism, foraging and dominance. We will present representative examples that highlight the techniques that may be employed when researching these areas, as well as their limitations.

Mate selection

Mate selection in animals is a well-studied area of behavioural ecology research. It is focused on

understanding the factors or recognition facets, such as correct species, sex, genetic relatedness and genetic quality that can lead an animal to select one individual over another for mating and/or pair bonding (Sherman *et al.* 1997). Laboratory mate selection research attempts to dissect the specific features of individuals that may make them attractive or unattractive as potential mates, and to identify what exact perceptual, physiological, and behavioural processes are involved in reaching the final mating decision.

Table 1. The costs and benefits of laboratory experiments in comparison with field studies. Vertebrate and invertebrate examples are provided.

<i>The Benefits of laboratory experiments in comparison with field studies</i>	<i>Examples</i>
Controlled settings allowing the manipulation of environmental variables.	Controlling water quality to modulate chemical cue concentrations in <i>Tilapia</i> (Gonçalves-de-Freitas <i>et al.</i> 2008).
Experimental control for testing the influence of single factors only.	Quality of artificial ant nests (Pratt 2005).
Controlled social development for captive-reared animals allowing assessment of innate responses and effects of social learning.	Tool-making in New Caledonian crows (Weir <i>et al.</i> 2002, Kenward <i>et al.</i> 2006).
Isolation and tracking of individual development and testing of reactions at an individual level.	Assessing effects of varying ontogeny on the quality of male zebra finches as perceived by female conspecifics (Spencer <i>et al.</i> 2003, 2005, Naguib & Nemitz 2007).
Ability to limit or exclude conspecific and/or heterospecific interactions.	Isolation of newly emerged <i>Polistes</i> females (Shellman & Gamboa 1982).
Ease of physiological manipulation for testing specific reactions.	Altering diet quality to assess effects on plumage characteristics and song development in zebra finches (Naguib & Nemitz 2007).
<i>The Costs of laboratory experiments in comparison with field studies</i>	<i>Examples</i>
Laboratory strains of animals are often domesticated and therefore may behave differently from their wild counterparts.	Physiological condition and mate preference behaviour in zebra finches (Ewenson <i>et al.</i> 2001, Rutstein <i>et al.</i> 2007).
Artificial food supply can alter foraging behaviour.	<i>Ad libitum</i> food sources lead animals to engage in more costly behaviours (Kotiaho 2001).
Mate selection experiments often do not allow subject and stimuli interaction to accurately assess subject motivation.	Spatial association measures in zebra finch mate preference choice trials (Rutstein <i>et al.</i> 2007).
Captive environments may increase subject and stimuli stress levels modulating behavioural results.	Mate preferences in zebra finches (Roberts <i>et al.</i> 2007).
Artefacts of the experimental environment can influence behavioural choices.	Leg bands and lighting conditions in zebra finch mate preference trials (Cuthill <i>et al.</i> 1997, Hunt <i>et al.</i> 1997).
Specific experimental results can be context-dependent and the unnatural laboratory environment can potentially obscure their observation.	Studies of kin recognition in the paper wasp, <i>Polistes dominulus</i> (Starks <i>et al.</i> 1998).

Through examples taken from a model system, the zebra finch (*Taeniopygia guttata*) (Zann 1996), we discuss here the use of live stimuli, highlighting findings and limitations of the laboratory setting, including impacts of experimental artefacts and proximal environmental factors on subject ontogeny.

The zebra finch is a small, sexually dimorphic, socially monogamous, Australian passerine. Mate preferences are frequently assessed via the presentation to a subject individual of a selection of live stimuli birds that vary to different degrees in phenotypic or behavioural attributes (Zann 1996), such as bill colour (e.g., Collins *et al.* 1994) or song variations (Houtman 1992). These preferences for one individual over another (or several others) in a simultaneous choice scenario are frequently measured by the proportion of choice time spent with a certain stimulus individual (reviewed in Forstmeier & Birkhead 2004), a measurement of passive choice (c.f. active operant choice: e.g., Riebel 2000). This passive spatial association method is widely used across different choice experiments (Hauber *et al.* 2000) but is not without interpretational constraints; the use of a sole preference metric and a lack of physical interactions between subject and stimuli can restrict determination of sexual or social subject motivation (Rutstein *et al.* 2007). The passive choice paradigm does not require context-specific action by the individuals beyond spatial movement, which occurs throughout everyday activities (Hauber *et al.* 2001) and for highly social birds such as the zebra finch, choice could just be indicative of a wish to associate rather than an actual sexual preference (Riebel 2000).

An alternative method to assess preferences is by measuring multiple behaviours displayed by the subjects that are naturally produced in specific contexts, such as female or male courtship displays (e.g., Burley *et al.* 1982, ten Cate 1985, Clayton 1990, Collins *et al.* 1994, Mansukhani *et al.* 1996). These may reveal social, sexual, or pair bonding preferences that spatial associations fail to show (Campbell & Hauber 2009). A further methodological option for confirming sexual motivation is to allow interactions between subject and stimuli in a free-flight aviary paradigm and use detailed behavioural observa-

tions to document sexual pair bonds formed, or ultimately, breeding success (e.g., Burley 1981, ten Cate 1985, Mansukhani *et al.* 1996, Rutstein *et al.* 2007). Most studies would benefit from a combination of assessment methodologies or a validation of the behavioural metric used in a series of studies to be able to comprehensively measure responses to specific traits in addition to confirming subject motivation and choice context (Rutstein *et al.* 2007).

In addition to mate selection based on varying phenotypic and behavioural traits, artefacts of the experimental environment may also impact on choice decisions. Examples include human alterations of the study species' appearance, such as the application of leg-bands that significantly alter zebra finch attractiveness (Cuthill *et al.* 1997), or factors such as the lighting environment where the absence of ultraviolet wavelengths important in avian vision may influence natural mating preferences (Hunt *et al.* 1997). The physiological stress hormone levels of both the subjects and the stimuli can also influence female choice behaviour, which is important to consider in the handling of subjects and giving time to acclimatise to novel testing environments (Roberts *et al.* 2007). However, physiological measures of stress responses, for example after the experimental separation of pair bonded mates, could in some cases provide critical insights into behaviourally cryptic discrimination abilities of individuals (Remage-Healey *et al.* 2003).

Furthermore, it is important to consider how proximate environmental factors may influence subject and stimulus ontogeny. For example the nutritional and stress-related hormonal environment can decrease the quality of males by altering ornamental plumage and song complexity, making them unattractive to females (Spencer *et al.* 2003, 2005, Naguib & Nemitz 2007). A poor developmental history of stimuli, for instance, may influence zebra finches' selections, irrespective of the phenotypic trait being experimentally manipulated, and poor developmental history of subjects may bias the strength, consistency or direction of their preferences based on their self-perceived poorer quality (Burley & Foster 2006). These factors indicate the critical importance of knowledge of developmental history when

testing for mate preferences in a laboratory setting. Finally, it must be considered that although laboratory environments permit experiments not otherwise possible in the field, captive environments may affect the physiology and behaviour of research animals leading to different responses than what would be found in their wild counterparts (e.g., Ewenson *et al.* 2001, Rutstein *et al.* 2007).

Laboratory settings, with zebra finches and with many other species, are ideal for measuring individual subject response to specific varying traits that cannot be assessed in the field, but can present difficulties in determining subject motivation. The artificial experimental environment may also modulate behaviours that would be present in the study species' natural habitat, which needs to be considered for result interpretation.

Nepotism

Natural selection acts on all living organisms, selecting for alleles and causing them to increase in frequency between subsequent generations. While selection typically occurs on individual survival to reproduction and the success with which individuals pass their own genes on into the next generation, non-descendant kin also share genes with the focal individual in proportion to their relatedness. Therefore, natural selection can select for individually costly behaviours that are preferentially directed at and favour relatives, as long as the benefit to kin (adjusted for relatedness) outweighs the cost to the focal individual (Hamilton 1963, 1964). In order for kin selection to act, individuals must be able to direct their aid to kin over randomly related (i.e. non-kin) conspecifics in the population. Within the laboratory setting, experiments can be performed to determine whether individuals can and do distinguish kin from non-kin, how they do so, and under what circumstances they show behavioural discrimination (Mateo 2002).

To direct aid towards kin, an individual must first be capable of reliably recognising or associating with kin. Individuals can be given choices with options that allow them to aid or harm partners that are kin or non-kin, allowing the

researcher to discern what features are used for kin recognition by removing or altering cues. In addition, the laboratory setting allows for detailed manipulation of the context of interactions. Frequently, animals exhibit different levels of kin-discrimination in different experimental contexts (reviewed in Liebert & Starks 2004) and these manipulations allow researchers to determine what external variables can affect kin-discrimination.

Neutral arena trials are frequently used in the laboratory to detect kin recognition. Two or more individuals are placed in an arena with minimal natural cues, then either resources (e.g., rove beetle larvae *Aleochara bilineata*: Lize *et al.* 2006) or threats (e.g., salamander *Hemidactylium scutatum*: Harris *et al.* 2003) may be added to observe competition or defence behaviour that favours related individuals.

Studies with contrived contexts can be used to test how nestmate-recognition (a correlate of kin recognition) ability is acquired, as well as the mechanisms and contexts of recognition. To test the context dependence of recognition, proximate environmental cues such as nest fragments can be introduced to arena trials to observe the interactions that result (e.g., the paper wasp *Polistes dominulus*: Starks *et al.* 1998). The learning of kin-recognition can be tested by removing very young animals such as paper wasps (*P. fuscatus*), from their parental nests upon emergence and raising them in controlled isolated environments (e.g., single boxes), then subsequently assessing their nestmate recognition abilities (Shellman & Gamboa 1982). These animals may also be reintroduced to unrelated conspecifics or nest material to determine whether they learn those cues from their environment and discriminate in favour of their "adoptive" kin (e.g., *P. carolina* and *P. fuscatus*: Pfennig *et al.* 1983).

In colony-living organisms, kin discrimination can be tested by introducing animals to laboratory colonies (e.g., Argentine ant *Pachycondyla luteipes*: Kikuchi *et al.* 2007). By varying the animals introduced (e.g., Kikuchi *et al.* 2007) or the circumstances of the colonies to which they are being introduced (e.g., ant *Linepithema humile*: Vásquez & Silverman 2008), the researcher can determine the contexts that lead to acceptance and rejection of nestmates

and non-nestmates. These laboratory studies have provided insight into the adaptive value of kin-recognition by increasing our understanding of when animals do and do not display kin-discrimination.

One major flaw with laboratory studies of nepotism is that kin-recognition and discrimination behaviours are frequently context dependent (e.g., Liebert & Starks 2004). In some species, kin-recognition can be as simple as treating all conspecifics in the colony or nest as kin (reviewed in Holmes & Sherman 1982). Additionally recognition behaviours are often affected by the social environment (e.g., Fletcher & Blum 1983) or by situational cues (e.g., Starks *et al.* 1998). Therefore, since context can be influential, the unnatural environment of the laboratory can potentially obscure important aspects of kin discrimination or even prevent it from being observed at all (e.g., Starks *et al.* 1998).

One method of partially addressing the lack of an appropriate fitness context in laboratory studies is to augment laboratory kin recognition studies with parallel experiments in the field (Kikuchi *et al.* 2007). Nestmate discrimination studies can be supplemented by studies of relatedness (e.g., ant *Proformica longiseta*: Seppä *et al.* 2008) and aggression (e.g., *Polistes fuscatus*: Gamboa *et al.* 1991) in the field to determine whether animals are discriminating in the contexts and ways that are predicted. In addition, context can be varied within the laboratory setting (e.g., Starks *et al.* 1998) to test for potential effects of a more natural context. Especially when combined with field studies and genetics, laboratory studies can provide valuable insight into the adaptive value and ontogeny of kin discrimination.

Foraging

All living organisms require food for energy to survive, and evolutionary optimal foraging theories predict decisions to be made during foraging that maximise energy intake (Drickamer *et al.* 2002). There is an extensive variety of foraging tests that can be carried out in a laboratory setting to investigate food preferences, foraging strategies, and the development of foraging proficiency. We present here select examples from

research on New Caledonian crows (*Corvus moneduloides*) of how behavioural ecology laboratory research can be used to enhance field findings and provide greater insight into the development and learning of foraging techniques.

New Caledonian crows are omnivorous, forest-dwelling corvids endemic to New Caledonia where field observations have shown these birds to be highly specialised in their manufacture of stick and hooked or barbed tools (exclusively from *Pandanus* spp. plants) that are used to pry out prey from holes (Hunt 1996, Hunt & Gray 2002). To expand on field findings, researchers in a controlled laboratory setting have specifically explored the development of tool use, the influences of social context, and the limitations of the tool use behaviour. Behavioural observations, through the tracking of a particular individual over time, have documented rapid *de novo* shaping and use of novel material (e.g., a piece of straight wire) to obtain food (Weir *et al.* 2002) in the absence of extensive prior experience in tool shaping, thus demonstrating spontaneous tool-making behaviour (Weir *et al.* 2002). Further developmental research revealed that laboratory-reared juveniles with controlled ontogeny and no prior experience, observation, or other means of social transmission of information, spontaneously used tools to obtain food (Kenward *et al.* 2005), which provided evidence of an innate predisposition for the tool-use behaviour.

The captive environment did, however, severely restrict sample sizes (e.g., Weir *et al.* 2002, one naïve individual of the two captive subjects) which limited the conclusions able to be drawn. In addition, the effects of the surrounding environmental conditions that would be present in the crows' natural habitat but missing in a laboratory setting must be taken into consideration. Field observations revealed that preferential stick or *Pandanus* tool use are highly specialised at the level of the individual (Hunt & Gray 2007) and between different locations (Hunt & Gray 2003). These levels of variability are consistent with a degree of niche partitioning and social transmission important for the cumulative evolution of the tool-use behaviour and that is not accounted for in a laboratory setting, which may modulate behavioural patterns (Hunt *et al.* 2007).

Furthermore, while there is evidence for a predisposition for stick tool manufacture, there is little complimentary evidence for innate recognition of the *Pandanus* plant (Kenward *et al.* 2005) over other potential tool materials, which has implications for substrate use when investigating tool manufacture in the laboratory and differential strengths in the role of social learning.

To investigate in detail the possibility of social transmission of information and effects of learning, the full ontogeny of the tool-use behaviour was documented (Kenward *et al.* 2006). Specifically, juveniles trained with human demonstrators later showed greater tool-use proficiency than the naïve individuals and preferred to handle objects they had previously seen being handled by their human trainers (Kenward *et al.* 2006). These and other findings suggest the specialised tool-using behaviour presents a partially inherited basis followed by individual learning and social influences to perfect tool formation and use (Kenward *et al.* 2005, 2006, Hunt *et al.* 2007) of which the full extent of social behavioural modulation is likely impossible to document in a captive environment.

Such laboratory experiments demonstrate the type of ontogenetic knowledge that can be gained about complex foraging strategies from socially isolated individuals and controlled rearing environments. Nonetheless, the natural ecological context has to be considered and how the artificial environment may modulate results and limit interpretations. Foraging studies such as these presented can benefit from a combination of both laboratory and field studies to have a greater contextual understanding of the observed behaviours.

Dominance

Many social animals exhibit dominance hierarchies that can be mutually beneficial through reducing conflicts among members of social groups involving the distribution of food (e.g., Appleby 1980), opportunities for reproduction (e.g., Blatrix & Herbers 2004), or the delegation of communal tasks (e.g., Tentschert *et al.* 2001). Since dominance hierarchies mediate factors that are necessary for survival and reproduc-

tion (e.g., Pagel & Dawkins 1997), the methods by which dominance hierarchies are established and maintained are important for understanding social behaviour in many animals. Within the laboratory setting, both the initial establishment of dominance hierarchies and the interactions within established dominance hierarchies can be observed. The value of dominance to an individual in a hierarchy can also be determined by manipulating environmental or group factors to see whether individuals choose to accept subordinate status, challenge for dominance, or leave the group.

Experimental trials with two or more individuals grouped in a neutral arena, and allowed to contest for dominance (e.g., *Polistes dominulus*: Tibbetts & Dale 2004, tilapia *Oreochromis niloticus*: Gonçalves-de-Freitas *et al.* 2008) allow researchers to isolate environmental predictors of the hierarchy structure such as chemical cues (e.g., Gonçalves-de-Freitas *et al.* 2008) or predictors of dominance in individuals such as female mated status (e.g., *P. fuscatus*: Downing 2004).

The establishment and maintenance of dominance hierarchies can be investigated by creating contrived situations within the laboratory and observing the response of the animals. Animals with different traits, such as varying genetic strains, can be grouped in artificial colonies (e.g., rats *Rattus norvegicus*: Ely *et al.* 1997) or given the opportunity to found their own colonies (e.g., *Polistes fuscatus*: Downing 2004). These studies can allow researchers to determine the effects of specific changes in context on the foundation of dominance hierarchies (e.g., *R. norvegicus*: Duncan *et al.* 2006).

The fitness component of dominance can also be studied in the laboratory by modifying the social environment within a group and observing the changes in the dominance hierarchy. This can involve removing the dominant individual(s) and observing the re-establishment of the dominance hierarchy after their removal (e.g., ant *Dinoponera quadriceps*: Monnin & Peeters 1999, *Polistes dominulus*: Strassman *et al.* 2004). The dominant individual(s) may then be replaced to observe how they fare in the newly established dominance hierarchy (e.g., ant *Harpagoxenus sublaevis*: Bourke 1988). In addition to behav-

ioural metrics, physiological measures such as sympathetic nervous system activity can be taken from individuals of differing dominance statuses to assess specific physiological correlates of being dominant or subordinate (Ely *et al.* 1997). Additionally, other modifications can be made to the nest or the environment such as removing reproductive-destined eggs in eusocial paper wasps to observe the effects on aggression in the dominance hierarchy (Reeve & Nonacs 1992).

Laboratory experiments allow for detailed manipulations and observations of social structures that are rarely possible in the field. However, laboratory experiments can exclude contextual cues that are important for the establishment and maintenance of a dominance hierarchy. Partners chosen by researchers are unlikely to be the same partners that would have been paired in the field, and may have different interactions. Laboratory studies that may have lacked important cues can be supplemented by replicating the observations in field studies of early dominance contests (e.g., *Polistes dominulus*: Dapporto *et al.* 2006) and by genetic studies of dominance in established colonies (e.g., paper wasp *Polistes annularis*: Peters *et al.* 1995). When these considerations are taken into account, however, laboratory studies and the creation of unnatural experimental situations can allow observations on and provide information about the establishment and maintenance of dominance hierarchies that cannot be gathered in field studies.

Laboratory contexts

Within a laboratory setting, behavioural experiments can be executed in any of three contexts: neutral, natural or contrived. While no laboratory experiment can perfectly replicate field conditions, the different contexts vary in the amount of control they offer and in how well they relate to field conditions.

A neutral context is an experiment in which the animal is removed from natural cues and placed in a simple neutral arena. While in the arena, the animal has very few cues outside of those specifically provided for the experiment. These experiments are artificial and far removed from natural conditions, but permit close obser-

vation, fine control of variables and detection of response to selected stimuli that are expected to provoke a reaction including, for example, a conspecific, a food item, or a predator cue.

Neutral arena trials with conspecifics can be used to study a variety of behavioural systems such as mate choice (e.g., cockroach *Blattella germanica*: Lihoreau *et al.* 2007, parasitic wasp *Aphidius ervi*: Villagra *et al.* 2007), kin selection (e.g., halictine bee *Lasioglossum erythrum*: Kukuk & Crozier 1990), dominance hierarchies (e.g., *Polistes dominulus*: Tibbetts & Dale 2004, *Oreochromis niloticus*: Gonçalves-de-Freitas *et al.* 2008) or food preferences (e.g., crayfish *Cherax destructor*: Meakin *et al.* 2008). They can also be used to study the mechanisms (e.g., Gravel *et al.* 2004), ontogeny or evolutionary history (e.g., Kukuk & Crozier 1990) of behavioural traits by presenting carefully controlled stimuli. But since the arenas are artificial environments they lack the variety of cues that animals may normally use, and may themselves provide cues that modulate the animal's behaviour. In addition, they remove context that may be important for making decisions, such as the distance to home or other resources, or the presence of predators. As such, they may not detect behaviour that would naturally be present (e.g., Starks *et al.* 1998) or may cause behaviour that would not normally occur (Weir *et al.* 2002). In order to compensate for the artificial nature of the environment, neutral arena trials should be followed with trials in more natural environments to confirm their findings. Despite these concerns, neutral arenas allow for very tight control over many variables, and can tease apart effects of interwoven factors.

A natural context experiment, in comparison, is an attempt made within the laboratory to replicate some aspect of a natural environment as much as possible. While a laboratory environment can never truly replicate field conditions, this style of experiment attempts to bridge the gap between field and laboratory experiments, by providing much of the control of a laboratory experiment with some of the realism of a field experiment.

Behavioural observations are frequently applied to natural contexts to document behaviours such as foraging or mate-choice (e.g., *Blat-*

tella germanica: Lihoreau *et al.* 2007). Observing the time spent performing different behaviours can suggest what factors are limiting to the reproductive success of an organism. In this way, behavioural observations may give insight into the adaptive value of different behaviours. They can also be used to study ontogeny by observing how behavioural patterns change over the lifespan of individual animals. While behavioural observations carried out in laboratories can add significantly to the range of observable behaviour, the behaviours may differ from those observed in the wild. Animals supplied *ad libitum* food, for example, may engage more frequently in energetically costly behaviours (Kotiaho 2001) and spend less time foraging.

A laboratory invertebrate nesting colony is an example of a natural context that can be manipulated by removing or adding individuals or brood (e.g., *Polistes fuscatus*: Reeve & Nonacs 1992) or altering the nest itself (e.g., ant: *Temnothorax curvispinosis* Pratt 2005). This then allows for detailed manipulations of one factor of the social environment or microhabitat of the animals and can be used to observe habitat selection (Pratt 2005), changes in the dominance hierarchy or changes in interactions among animals (e.g., Nonacs *et al.* 2004).

However, nests kept within a laboratory setting will experience a different environment from those in a field setting and animals may respond differently to nest manipulations in the presence of abundant food and the absence of predators and conspecifics. Laboratory nest manipulations therefore, can provide a first step for testing hypotheses but benefit from being supplemented by observations and complementary experiments carried out in a field setting.

Finally, in a contrived context experiment, the researcher is neither trying to replicate a natural environment nor make the environment as neutral as possible. Instead the researcher provides an artificial environment with novel cues that do not resemble the natural environment. Contrived context experiments can be excellent for determining the factors of the environment that have the largest impact on particular behaviours. For example, an organism can be introduced to an entirely novel environment where their reactions can be used to provide information about their

environmental perceptual processing. Novel environment experiments are frequently used to study foraging behaviour (e.g., honeybees *Apis mellifera ligustica*: Sanderson *et al.* 2006) as many animals will take food from novel sources, or they can test the ability of organisms to distinguish among different food sources (e.g., Sanderson *et al.* 2006), or to secure other resources such as nesting material or nest locations. However, it is difficult to know whether animals perceive and interact with an artificial stimulus (e.g., a honeybee feeder) in the same way they do with a natural stimulus (e.g., a flower).

These studies can be used to determine the mechanisms organisms use to make decisions. They can also be applied to study the ontogeny of decision-making by exposing an organism to novel environments at different points in its life-cycle. The major concern with studies using novel environments is that the environment may be so remote from natural as to make the results inapplicable. Novel stimuli may also leave out important cues, such as scents or polarised light and similar studies should be carried out presenting natural stimuli to see if the same responses are observed.

Conclusions

In this review, we have detailed select questions of behavioural ecology research to which extensive laboratory methods have been applied, to understand the evolutionary and social context of animal behaviour in vertebrates and invertebrates. Different laboratory contexts have their advantages and disadvantages and none of them are complete alone. Laboratory experiments can be greatly informative for explaining the behavioural patterns observed in the field and can provide extensive information on details of behaviours that cannot be tracked in other ways. Despite their experimental and ontogenetic appeal, such laboratory experiments cannot stand alone. In general, laboratory experiments have more control but less realism than field experiments (Underwood 2009) and therefore the goal of the laboratory should alternatively be to better understand animal behaviour in the wild. Many of the best experimental progres-

sions of behavioural research take work carried out in the field and elaborate on it in the laboratory or vice versa. Our literature survey suggests that future studies should focus on combining both field and laboratory based data to increase theoretical appeal and experimental validity.

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