Convocatorias 2016
Proyectos EXCELENCIA y Proyectos RETOS
Dirección General de Investigación Científica y Técnica
Subdirección General de Proyectos de Investigación

AVISO IMPORTANTE

En virtud del artículo 16 de la convocatoria **NO SE ACEPTARÁN NI SERÁN SUBSANABLES MEMORIAS CIENTÍFICO-TÉCNICAS** que no se presenten en este formato.

Es obligatorio rellenar los tres apartados (A, B y C). La parte C de la memoria no podrá exceder de 20 páginas.

Lea detenidamente las instrucciones para rellenar correctamente esta memoria, disponibles en la web de la convocatoria.

Parte A: RESUMEN DE LA PROPUESTA/SUMMARY OF THE PROPOSAL

INVESTIGADOR PRINCIPAL 1 (Nombre y apellidos)**:**

Dr. Wilhelmus (Pim) Edelaar

INVESTIGADOR PRINCIPAL 2 (Nombre y apellidos)**:**

TÍTULO DEL PROYECTO: "Causas y consecuencias de la Elección de Hábitat Coincidente, una vía alternativa hacia la adaptación evolutiva"

ACRÓNIMO: HabitatMatching

RESUMEN Máximo 3500 caracteres (incluyendo espacios en blanco):

Desarrollos teóricos recientes y datos empíricos propios demuestran que las poblaciones pueden adaptarse cuando los individuos eligen el hábitat en el que mejor rendimiento tienen – desafiando el paradigma actual que la adaptación de las poblaciones es impulsado exclusivamente por la selección natural. Esta hipótesis plausible de 'Elección de Hábitat Coincidente' aún no se ha confirmado plenamente. Una mejor comprensión del proceso de adaptación puede tener un gran impacto sobre nuestro medio ambiente, sociedad y economía. Por tanto, el objetivo del proyecto es aumentar nuestra comprensión de la adaptación al documentar y comprender las causas y consecuencias de Elección de Hábitat Coincidente. Al exponer individuos de la mosca de fruta (Drosophila melanogaster) a hábitats contrastantes y al permitirles elegir, por primera vez vamos a estudiar los efectos de Elección de Hábitat Coincidente en una amplia gama de temas clave, como el cambio de nicho, la especiación, los efectos sobre la comunidad biológica, y la fisiología del estrés. La manipulación experimental de los fenotipos individuales es el enfoque fundamental, ya que distingue a Elección de Hábitat Coincidente de diferentes formas de la elección del hábitat mejor conocidas, tales como los genes de preferencias de hábitat y la impronta. Un número de técnicas y recursos modernos de la neurobiología y de la biología del desarrollo se emplearan de forma creativa para manipular los fenotipos de la mosca en esta arena eco-evolutiva. Al seleccionar poblaciones de moscas para altos/bajos grados de Elección de Hábitat, tenemos la capacidad de testar por el efecto específico de Elección de Hábitat Coincidente. A nivel teórico se estudiará la evolución y las consecuencias de Elección de Hábitats Coincidentes. Los impactos esperados incluyen la publicación de artículos novedosos de alta calidad, la clarificación/desarrollo de conceptos evolutivos

existentes/nuevos, la formación de jóvenes científicos, y la introducción de nuevas técnicas en el campo. Este interesante proyecto está diseñado para mejorar la forma de pensar acerca de la evolución y la adaptación, y actuar como un catalizador para futuros estudios en los campos relacionados como no-relacionados (incluso aplicados).

PALABRAS CLAVE: Elección de Habitat Coincidente, líneas de selección, Drosophila, divergencia genética adaptativa, evolución del nicho.

TITLE OF THE PROJECT: "Causes and consequences of Matching Habitat Choice, an alternative mechanism of evolutionary adaptation"

ACRONYM: HabitatMatching

SUMMARY Maximum 3500 characters (including spaces):

Recent theoretical developments and own empirical data show that populations can adapt when individuals choose habitats in which they perform best – challenging the current paradigm that adaptation of populations is exclusively driven by natural selection. This plausible hypothesis of ´Matching Habitat Choice´ has yet to be fully confirmed. A better understanding of the process of adaptation can have major impacts on our environment, society and economy. The objective of the project is therefore to increase our understanding of adaptation by documenting and understanding the causes and consequences of Matching Habitat Choice. By exposing individual Fruit flies to contrasting habitats and allowing them to choose, for the first time we will study the effects of Matching Habitat Choice on a wide range of key topics like niche shift, speciation, community effects, and stress physiology. Experimental manipulation of individual phenotypes is the fundamental approach, as it distinguishes Matching Habitat Choice from better known, different forms of habitat choice such as habitat preference genes and imprinting. A number of modern techniques and resources from developmental and neurobiology are creatively employed to manipulate fly phenotypes in this eco-evolutionary arena. By selecting fly populations for high/low degrees of Matching Habitat Choice, we will be able to test for the specific effect of Matching Habitat Choice. At the theoretic level we will study the evolution and consequences of Matching Habitat Choice. Expected impacts include publication of high-quality ´eye-opening´ major papers, clarification/development of existing/new theory and evolutionary concepts, training of young scientists, and introduction of novel techniques into the field. This exciting project is designed to improve the way people think about evolution and adaptation, and to act as a catalyst for future studies within related and even unrelated (incl. applied) fields.

KEY WORDS: Matching Habitat Choice, selection lines, Drosophila, adaptative genetic divergence, niche evolution, speciation.

Parte B: INFORMACIÓN ESPECÍFICA DEL EQUIPO

B.1. RELACIÓN DE LAS PERSONAS NO DOCTORES QUE COMPONEN EL EQUIPO DE TRABAJO (se recuerda que los doctores del equipo de trabajo y los componentes del equipo de investigación no se solicitan aquí porque deberán incluirse en la aplicación informática de solicitud). Repita la siguiente secuencia tantas veces como precise.

1. Nombre y apellidos: Adrian Baños-Villalba
   Titulación: MSc in Biodiversity and Conservation Biology (University Pablo de Olavide)
   Tipo de contrato: PhD student directed by Dr. Edelaar, financed by MINECO (former FPI)
   Duración del contrato: until 15 January 2018
   Adrian´s thesis focuses on Matching Habitat Choice in a wild study system (grasshoppers). His experience and ongoing research will be a good background for the new PhD student involved with the experiments with the Fruit flies.

2. Nombre y apellidos: Juan Ramón Peralta Rincón
   Titulación: MSc in Biology and Biodiversity Conservation Biología (University of Salamanca)
   Tipo de contrato: directed by Dr. Edelaar, inscribed in the PhD program, without funding
   Duración del contrato: not applicable
   Juan Ramón has just started to work with Fruit flies, and the idea is to already get started with the design and testing of habitat choice cages and the collection of some preliminary behavioural, physiological and reproductive data. Depending on how he does (so far he looks like a keen and sharp student), he will be a good candidate for the PhD position that we apply for.

3. Nombre y apellidos: Graciela Escudero
   Titulación: MSc in Animal Ecology (University of Groningen, The Netherlands)
   Tipo de contrato: former technician and lab coordinator, paid from project of Dr. Edelaar funded by MINECO, and probably hired again in the near future as technician for the Explora project.
   Duración del contrato: not known, possibly Oct. 2016-Dec. 2017.

B.2. FINANCIACIÓN PÚBLICA Y PRIVADA (PROYECTOS Y/O CONTRATOS DE I+D+I) DEL EQUIPO DE INVESTIGACIÓN (hasta un máximo de 10 proyectos y/o contratos).

PARTICIPATING INVESTIGATOR: Dr. Pim Edelaar
PROJECT TITLE: '*Puede producirse la adaptacion por otro mecanismo que por la seleccion natural? Una prueba experimental* ´
PRINCIPAL INVESTIGATOR: Dr. **Pim Edelaar**
FINANCING ENTITY: proyecto EXPLORA, Ministry of Economy and Competitiveness, Spain
LENGHT FROM: 01/01/2015 – 31/12/2016
FINANCING RECEIVED (en euros): 67,000
RELATION TO CURRENT PROJECT: very related (*we test if we can enforce experimental speciation in a highly social, group-living vertebrate (Zebra finch) by electronically/mechanically manipulating functional traits and resources in space*)

PARTICIPATING INVESTIGATOR: Dr. Pim Edelaar
PROJECT TITLE: '*European Network on Invasive Parakeets: Understanding Invasion Dynamics and Risks to Agriculture and Society (ParrotNet)* ´
PRINCIPAL INVESTIGATOR: Dr. Jim Groombridge
FINANCING ENTITY: COST Action; European Commission & European Science Foundation (ESF)
LENGHT FROM: 01/10/2013 – 30/09/2017
FINANCING RECEIVED (en euros): 300,000
RELATION TO CURRENT PROJECT: not related

PARTICIPATING INVESTIGATOR: Dr. Pim Edelaar
PROJECT TITLE: '*Neglected Mechanisms of Adaptation to a Changing World* ´
PRINCIPAL INVESTIGATOR: Dr. **Pim Edelaar**
FINANCING ENTITY: Plan Nacional, Ministry of Economy and Competitiveness, Spain
LENGHT FROM: 01/01/2013 – 31/12/2015
FINANCING RECEIVED (en euros): 187,000
RELATION TO CURRENT PROJECT: very related (*as part of this project we tested for matching habitat choice in grasshoppers; more details in the text and Figures 1-4*)

PARTICIPATING INVESTIGATOR: Dr. Pim Edelaar
PROJECT TITLE: '*Evolution of Invasiveness in Exotic Species: Mechanisms and Implications* ´
PRINCIPAL INVESTIGATOR: Dr. **Pim Edelaar**
FINANCING ENTITY: Program Ramón y Cajal, Ministry of Science and Innovation, Spain
LENGHT FROM: 01/06/2012 – 30/06/2017
FINANCING RECEIVED (en euros): 180,000
RELATION TO CURRENT PROJECT: somewhat related

PARTICIPATING INVESTIGATOR: Dr. Pim Edelaar
PROJECT TITLE: '*Exploring the Potential of Zebra finches for Experimental Speciation Research* ´
PRINCIPAL INVESTIGATOR: Dr. **Pim Edelaar**
FINANCING ENTITY: European Science Foundation (ESF – FroSpects Visitation Program)
LENGHT FROM: 07/03/2012 – 16/03/2012
FINANCING RECEIVED (en euros): 1,000
RELATION TO CURRENT PROJECT: very related (*a pilot experiment that led to the development of the Explora project*)

PARTICIPATING INVESTIGATOR: Dr. Pim Edelaar
PROJECT TITLE: '*Assessing Contemporary Evolution in Invasive Species*'

PRINCIPAL INVESTIGATOR: Dr. **Pim Edelaar** and Dr. José L. Tella (Estación Biológica de Doñana)
FINANCING ENTITY: Program JAE-Doc, CSIC, Spain
LENGHT FROM: 15/09/2009 – 31/05/2012
FINANCING RECEIVED (en euros): 100,000
RELATION TO CURRENT PROJECT: somewhat related

PARTICIPATING INVESTIGATOR: Dr. Pim Edelaar
PROJECT TITLE: '*Las Aves Exóticas Invasoras como Transmisoras de Patógenos: Enfermedades Emergentes y Procesos de Selección en los Hospedadores*'
PRINCIPAL INVESTIGATOR: Dr. José L. Tella (Estación Biológica de Doñana)
FINANCING ENTITY: Proyecto de Excelencia, Consejería de Educación y Ciencia, Junta de Andalucía, Spain
LENGHT FROM: 01/01/2009 – 31/12/2012
FINANCING RECEIVED (en euros): 190,000
RELATION TO CURRENT PROJECT: not related

PARTICIPATING INVESTIGATOR: Dr. Pim Edelaar
PROJECT TITLE: '*Una Aproximación Multidisciplinar al Estudio de las Invasiones Biológicas: el Caso de las Aves Exóticas en Andalucía*'
PRINCIPAL INVESTIGATOR: Dr. Fernando Hiraldo (Estación Biológica de Doñana)
FINANCING ENTITY: Proyecto de Excelencia, Consejería de Educación y Ciencia, Junta de Andalucía, Spain
LENGHT FROM: 01/01/2008 – 31/12/2011
FINANCING RECEIVED (en euros): 200,000
RELATION TO CURRENT PROJECT: not related

PARTICIPATING INVESTIGATOR: Dr. Antonio Prado
PROJECT TITLE: '*Los movimientos oculares microsacaídos: generación, control y valor diagnóstico*´
PRINCIPAL INVESTIGATOR: Dr. Miguel Escudero González
FINANCING ENTITY: Proyecto de Excelencia, Consejería de Educación y Ciencia, Junta de Andalucía, Spain
LENGHT FROM: 01/01/2008 – 31/12/2011
FINANCING RECEIVED (en euros): 196,000
RELATION TO CURRENT PROJECT: not related

PARTICIPATING INVESTIGATOR: Dr. Antonio Prado
PROJECT TITLE: '*Efectos de la contaminación magnetica usando* Drosophila melanogaster *como modelo de estudio*´
PRINCIPAL INVESTIGATOR: Dr. **Antonio Prado Moreno**
FINANCING ENTITY: Acciones Complementarias (Programa de Cooperación Internacional, Ministerio de Educación y ciencia)
LENGHT FROM: 01/01/2007 – 31/12/2008
FINANCING RECEIVED (en euros): 28,200
RELATION TO CURRENT PROJECT: not related (*but same study species*)

C.1. PROPUESTA CIENTÍFICA

THE CENTRAL TOPIC OF THIS STUDY

The evolutionary adaptation of living organisms to their environment is a central feature of life, and allows life to continue. The environment that any organism experiences can change for many reasons, some of them natural and others due to human actions, like climate change, changes in land use and agricultural practices, urbanisation, use of chemicals/pesticides/medicines, introduction to new parts of the world, etc. In some cases the capacity of populations to adapt is considered beneficial in terms of conservation of biodiversity (incl. ecosystem functioning and services) or in agricultural and industrial production systems. However, in other cases evolving populations pose threats to our society and economy, e.g. in the form of diseases, pests or invasive species. Therefore, understanding in greater detail how populations adapt and evolve is both of great fundamental and applied significance (Losos et al. 2013).

THE CURRENT CONCEPTUAL FRAMEWORK

Evolution is normally defined as a change in the genetic composition of a population. Adaptive evolution, which increases the mean performance of populations, is typically thought to be driven exclusively by natural selection: the fittest individuals survive, reproduce and copy the underlying genetic material coding for their greater fitness at higher rates than less fit individuals. As such, natural selection is the ultimate explanation for the diversity of life on this planet. Philosophers of science have called this basic idea of "evolution by natural selection" the single-most important scientific insight achieved by mankind. It has influenced society in many ways, not only in the biological, agricultural and other life sciences, but also in medicine, economics, sociology, psychology, theology, robotics, information technology (self-evolving algorhythms), etc., and both with respect to fundamental and applied aspects (Losos et al. 2013). It is often repeated that "*Nothing in biology makes sense except in the light of evolution*". But do we really understand evolution? There is a growing scientific call that evolutionary theory needs to be updated and broadened (Laland et al. 2014).

Besides natural selection, other processes can also lead to evolution, for example mutation, recombination, or gene flow (individual movement and settlement) among populations. However, these processes are only considered to influence the variation in genetic material available for natural selection to act upon: they do not have a directional effect towards greater adaptation. Hence, the current paradigm is that only natural selection can deterministically drive populations towards higher levels of adaptation (Edelaar & Bolnick 2012). Specifically, it is typically assumed that the movement and interchange of individuals among populations makes these populations to be more similar, such that more gene flow leads to the homogenisation of populations (just as when we keep moving some paint between a pot with black paint and a pot with white paint, we end up with two pots of grey paint). As such, natural selection and gene flow will act as antagonists in a variable environment: natural selection acts towards ecological divergence and local adaptation of populations, whereas gene flow acts towards the loss of divergence and local adaptation. These two main ideas, (1) that only natural selection can drive adaptative evolution and (2) that gene flow erodes it, are repeated over and over in scientific text books (Edelaar & Bolnick 2012).

CHANGING THE PARADIGM

The paradigm that natural selection is the only evolutionary force that can drive adaptation can be shifted, or at least adjusted: when dispersal movements by individuals depend on their local performance as determined by their genotype, the behaviour of individuals can also lead to the adaptation of populations (Ravigné et al. 2004, Edelaar et al. 2008, Holt & Barfield 2008, Ravigné et al. 2009, Bolnick & Otto 2013). A simple example explains this best. Imagine a population of birds with large or small bills, which can feed on large or small seeds. We, as well as the birds, know that individuals with large bills are better at cracking large seeds, and that individuals with small bills are better at picking up and handling small seeds. Small and large seeds may be found in different

habitats. If so, we then would expect that large-billed birds, after exploring the feeding habitats, would select the habitat with large seeds because there life is easier for them. Likewise, small-billed birds would select the habitat with small seeds. Hence, if we started with a mixed population of large-billed and small-billed birds, then the initial sampling and movement of individuals between the two types of habitats results in a final divergence into two subpopulations, each locally adapted to the food in their own environment. Some support for such a mechanism has been reported, including from our own studies (Bolnick et al. 2009, Edelaar et al. 2012). And this adaptation could happen even if no individual has died, and no individual has reproduced. In other words: we can observe adaptive evolution, but in the absence of natural selection (defined as variance in reproductive success due to differential survival or fecundity) (Edelaar & Bolnick 2012, Bolnick & Otto 2013). Here, the behavioural force driving adaptive evolution is a type of habitat choice called Matching Habitat Choice (Ravigné et al. 2004) (reviewed in Edelaar et al. 2008). A crucial difference with natural selection is that here individuals are not the *subjects* of (natural) selection <u>by</u> the environment, but they are the *agents* of selection <u>of</u> the environment. Basically, adaptive evolution does not necessarily always happen due to natural selection, but can also happen because of the avoidance of natural selection. In other words, populations are not being evolved, they evolve themselves, as an adaptive behavioural response to selective forces present in the environment.

We have recently developed a framework which integrates all mechanisms of adaptation, depending on whether adaptation happens due to demography (as in natural selection) or due to development (as in adaptive phenotypic plasticity), and whether it happens because the functional trait is changing or because it is the environment which is changing (Figure 1). This framework confirms, at least conceptually, that the choice of the environment plays a distinct role in adaptation and evolution. It also implies that it can have the same consequences as natural selection. It furthermore incorporates different types of habitat choice (by genetic preference, or by previous experience/familiarity, or by matching habitat choice) into a single evolutionary force, which we call "selection of the environment". It has been known for some time that habitat choice can play a powerful role in population divergence and maintenance of genetic diversity (Edelaar & Bolnick 2012, Edelaar et al. 2008, Ravigné et al. 2009, Bolnick & Otto 2013, Jones & Probert 1980, Webster et al. 2012, Rice & Salt 1988). Nonetheless, it is hardly recognised that habitat choice can do this independently of natural selection (even if it evolved because of natural selection). This is partly because the more traditional types of habitat choice (due to genetic preference or due to prior experience) only drive adaptation if the choice of habitat is somehow linked to a functional trait that enables adaptation. And this linkage can only occur if natural selection is acting simultaneously. Therefore, the effect of habitat choice is never considered to operate independently of natural selection, only in co-occurrence. This co-occurrence is not necessary for matching habitat choice, because it is the perceived level of performance and adaptation that directly influences the preference for a certain environment. In other words, preference for a certain environment is always linked with the relevant functional trait(s), so that Matching Habitat Choice can result in selection of the environment in a way that is independent of natural selection.

IMPORTANCE VERSUS ATTENTION FOR MATCHING HABITAT CHOICE

Because in principle Matching Habitat Choice can independently have the same effects on populations as natural selection, under certain circumstances Matching Habitat Choice can have impacts on a broad range of phenomena which are key topics in the fields of ecology and evolution: the rate and degree of local adaptation, the carrying capacity and stability of populations, the maintenance of genetic variation, the genetic divergence of populations, the evolution of ecological niches, the rate and success of adapting to or invading novel habitats, and the probability of speciation (Edelaar & Bolnick 2012, Edelaar et al. 2008, Bolnick & Otto 2013). It also appears to be a process whose occurrence seems likely, given the right circumstances. Therefore one would expect that this process has been studied in depth and is now well-understood. In contrast, only a handful of theoretical and empirical papers have dealt with this topic directly (reviewed in Edelaar et al. 2008, Ravigné et al. 2009, Bolnick & Otto 2013, Karpestam et al. 2012), with other papers giving indirect evidence or passing attention. Typically, studies use patterns of genetic divergence or local adaptation as evidence for natural selection, without considering Matching Habitat Choice at all (Kawecki & Ebert 2004). This may be partly because it goes against the mainstream view that gene flow homogenises populations, partly because it is often confused with unrelated forms of habitat choice (e.g. habitat preference genes (Jaenike & Holt 1991), imprinting (Davis & Stamps 2004)), and partly because of

the logistic challenges of confirming and studying it experimentally (Edelaar & Bolnick 2012, Edelaar et al. 2008).

PAST AND CURRENT PROJECT ON MATCHING HABITAT CHOICE

Even though Matching Habitat Choice has the potential to have an impact on a large range of key ecological and evolutionary topics, it has hardly been studied, especially empirically. I have taken up this challenge in two projects.

A. I was PI on an Excellence project funded by MINECO that finished December 2015. About half of this project was devoted to the study of matching habitat choice, involving research with ground-perching grasshoppers. These grasshoppers typically have the same colour as the soils on which they live, most likely because this increases crypsis and therefore reduces predation risk. Based on the literature, it was not clear if this adaptation was caused by natural selection or by adaptive phenotypic plasticity, or perhaps even by matching habitat choice. These hypotheses were addressed doing a range of studies and experiments in the field and in the lab. We have taken advantage of encountering at the beginning of the project an abandoned housing site where grasshoppers have colonised different types of urban pavement that differ in colour (see Figure 2). We saw that grasshoppers on e.g. dark asphalt roads have a different colour than grasshoppers occurring on the pale sidewalks. This is highly surprising, because these different pavements are only a few meters wide and occur alongside, while we also established here that marked grasshoppers move on average 12 meters per day. This means that populations should homogenise within a day, unless some mechanism maintains the observed divergence between pavements in grasshopper colouration (Figure 3). We have established that this is not due to selective predation (natural selection): computer simulation of selection suggests that mortality rates of up to 82% per day are needed, whereas capture-recapture modelling of marked grasshoppers show this to be only 3%. We also excluded adaptive plasticity as a cause, because while adults can adapt their colour to the local substrate over time, they do so very slowly. In fact, we also found evidence for a moderate to large genetic component to colour, suggesting that the population structuring between different types of pavement (dark asphalt road, pale sidewalk, brown bike path, dark grey foot path) represents a micro-geographic genetic population structure. Finally, we obtained positive evidence that grasshoppers select those pavements onto which they are most cryptic, most likely because they can assess the difference in colour between themselves and that of the substrate (Figure 4). When we painted grasshoppers a different colour, they made greater use of habitats that had that colour. When we injected grasshoppers with a hormone that made them darker, they again made greater use of darker habitat, both in the lab and in the field (asphalt road). Our results therefore not only establish that selection of the environment can aid in local adaptation and population genetic structuring, it even shows that it can be the main driver. On top of that we have data that suggests that such choice of environment also promotes positve assortative mating, and thereby increases the maintenance of genetic variation in colour across the metapopulation and enhances the local adaptation in colour in the subsequent generation.

We believe these results are of great interest. We used them to test the conceptual framework that we developed, because we can quantitatively address each of its components (we have no evidence for niche construction, and cannot imagine how grasshoppers could actively change the colour of the substrate on which they reside, so that one is also excluded). By combining these theoretical and empirical results, we think we can draw much attention to the phenomenon and importance of selection of the environment as an independent driver of adaptive evolution, and are therefore preparing a manuscript for the journal Nature. Of course we do not yet know if this ms will be accepted for publication here, but if not here we do think it will be so in other major journals, e.g. PNAS, PloS Biology or Ecology Letters. It has to be said that this possibility is not something that just occurred to us at some point: in the previous proposal we have written that if we obtained positive results in the planned experiments, the topic would lend itself for such a kind of publication. We have therefore refrained of publishing partial results in minor papers as the research progressed, because it is the synthesis of the separate elements that makes the story. Publication has also been delayed by the delay of the first payment of the project, more than 9 months late. (This also caused us to postpone the other part of the proposal for more than a year). Extension of the project was not possible because of support by FEDER funds, so 26,000 euros were returned at the end of the project.

Additional results from this study are also worth mentioning. We have detected for the first time the presence of feomelanin in an insect, whereas until recently it was believed that this pigment was restricted to terrestrial vertebrates only. Surprisingly, our grasshopper uses the same biochemical pathway as the type of feomelanin that is associated with Parkinson´s disease, which means that insects may be developed as a model organism to study this association. This was published in the journal Pigment Cell & Melanoma Research. We furthermore have established that the change in colour of grasshoppers to that of their substrate is improved under the risk of predation. This confirms the adaptive nature of this colour change, but also suggests there are unknown costs to this change which reduce colour change when risk is low. This result is resubmitted to the journal Behavioral Ecology. Another result which we consider sending to Proceedings of the Royal Society B is that grasshoppers that are moved between two types of habitat on a daily basis and that are only disturbed in one habitat, later on prefer to stay in the habitat without disturbance, but develop a colour that makes them cryptic in the habitat with disturbance. Paradoxically, this appears to be a maladaptive response because they reduce crypsis in their preferred habitat, but we see it as a kind of insurance, in case the preferred, safe habitat cannot be reached. We also plan to publish our breeding protocols, because we managed to keep and rear grasshoppers without the use of green plants, which seems to not have been achieved before.

For results and two further papers close to submission involving unplanned simulation studies of matching habitat choice I refer to research topic 6 further below.

B. I am also PI on an Explora project, also funded by MINECO (very competitive, only 1 out 13 projects from UPO and EBD-CSIC was awarded in that round). This should not be seen as an indication that research on matching habitat choice is inherently risky. What it does confirm is that the topic of the research is of great scientific interest and that the results have the potential for a large impact. What we aim to do in this project, is to turn a single flock of the highly-social Zebra finch into two separate populations, each locally adapted to its own resource, just because they employed matching habitat choice. And this separation, followed by a reduction of gene flow, would be achieved without any geographical barrier, in fact it would happen in a room of 5 by 6 meters. How would we do this? To create two types of resources to which individuals have to adapt, we have built automated feeders which only open and give access to food if an approaching individual carries on its leg a transponder with a code which activates the feeder: each type of feeder opens up to half of the individuals present. The transponders therefore can be seen as the ecological trait which influences local performance on the two distinct resources. By placing each type of feeder in an opposite part of the room (which will have some subdividing panels to make the travel distance a bit larger), we will force the birds to feed in a different part of space. Next, we will put up nest boxes close to the feeders, which hopefully will cause the birds to breed close to their feeding patch and thereby stimulate assortative mating among individuals with transponder codes from the same group. These codes will be initially assigned to individuals on the basis of neutral genetic markers (microsatellite length), such that we can calculate indices of population genetic structuring in the parent population, and the evolutionary response to divergent selection of the environment in the offspring generation. If assortative mating is perfect such that the transponder code that will be inherited by the offspring (based on their microsatellite lengths) makes them use the same feeders as their parents and therefore to remain in their own population, then one might say that we have reduced gene flow between these two groups to zero, and in fact we have created two reproductively isolated "species" of Zebra finch – in a room of 30 square meters, and driven by selection of the environment. The risky part of this research is that it is possible that the Zebra finches will not break up their social group, but will simply move from one feeding patch to the other as a single flock, or will not breed close to their feeder. There is no way to know until we try it out ...

We have no results yet because, unfortunately, also here delay is involved. The cages that we proposed to use for this experiment in the mean time have become unavailable, so we are now in the process of adapting two spaces specifically for this. This involves extensive remodelling (air control, preparation of all surfaces, building of a quarantine room and separate cages), because the spaces have to be officially accredited by the relevant authorities for the purpose of animal experimentation. We have therefore yet to start the experiments, although the feeders have been designed, tested, perfected and finished. On the positive side, the PhD student that we apply for here will also be exposed to this research project and it might even be included into his thesis, depending on involvement.

Overall then, we have achieved increased theoretical support for the importance of matching habitat choice (the novel framework), obtained important positive evidence that it plays a role in local adaptation and population structuring in the field (the results on grasshoppers colonising novel urban habitats), and expect it to have the potential to cause micro-scale population structuring, local adaptation and perhaps even reproductive isolation in a species which shows no comparable population structure at the scale of a continent (Zebra finches in Australia). We now need to reinforce these developments by additional dedicated demonstrations of the origins and impacts of matching habitat choice, preferably experimentally and by using a species that is easily manipulated and for which we know that matching habitat choice is operating. We propose to do this with Fruit flies selected for increased degrees of matching habitat choice.

OBJECTIVE OF THIS PROJECT

The overall objective of the research proposed here is *to document and understand the causes and consequences of Matching Habitat Choice*, thereby improving our understanding of evolutionary adaptation. Below this overall objective is broken down into six focused topics (1-6). This intentionally broad approach is chosen to maximise scientific impact and to stimulate future research. By showing the independent impact that Matching Habitat Choice can have on some important topics in ecology and evolution, we hope that people will recognise the benefit of taking this mechanism of adaptation into consideration.

A SUCCESSFUL APPROACH TO MATCHING HABITAT CHOICE – EXPERIMENTAL MANIPULATION OF PHENOTYPES

A hindrance to the study of Matching Habitat Choice (from now on: MHC) is the logistical challenge of studying it experimentally. Simply showing that individuals with different phenotypes prefer different habitats is not sufficient, as many other mechanisms can explain this finding, e.g. genetic variation for habitat preference, imprinting on certain habitats, competitive exclusion, etcetera[5]. The best way to exclude other interpretations is to vary the functional phenotype and make it independent of genotype and past experience, and see if this change in phenotype affects habitat choice, and in the predicted direction of increased fitness (Ravigné et al. 2004). This approach has been applied very rarely (Karpestam et al. 2012). As part of our previous project financed by MINECO, by using this approach we have obtained what we consider some of the best evidence for Matching Habitat Choice to date (Figure 3,4).

FRUIT FLIES AS A MODEL SPECIES FOR ECO-EVOLUTIONARY STUDIES

In this project we therefore continue this successful approach. However, we will change to a different species, the Fruit fly *Drosophila melanogaster*. This species has several important advantages. (i) They have a small size (few mm), allowing maintenance of larger numbers and easy replication of experiments, increasing sample sizes and statistical power. (ii) Their small size and short generation time (2 weeks) enable the establishment of selection lines (i.e. populations that have been selected for a certain trait; see for details below). We will select for lines with a high level of MHC, and for lines with a low/absent level of MHC. The use of individuals from the high MHC lines should give stronger effects in the experiments outlined below. It also gives certainty that we are testing for the effect of MHC because we use populations with proven well-developed MHC, improving interpretation of the results. Comparison with results obtained with individuals of the low MHC lines further improves insight and interpretation. (iii) There are valuable resources and techniques available for this species (see for details below) that can be used to manipulate functional phenotypes in different ways. Combined with their small size and ease of keeping, this means that we can produce and use independent, replicate selection lines for high or low MHC based on different phenotypic traits. The use of replicate lines gives greater generality to the results and greater statistical power. Specifically, to manipulate phenotypes we will apply some modern techniques and resources from developmental and neurobiology that have not been used before in an eco-evolutionary context, adding novelty to the proposed research.

SELECTION LINES

Selection lines are commonly produced in Fruit flies, for almost any kind of trait. The short generation time of two weeks for Fruit flies means that selection lines can be established within a short time (a few months) to obtain a marked genetic divergence between populations (Powell 1997). In fact, it was for this reason that the species has become such a popular laboratory organism. We will, as far as we know for the first time, establish selection lines for high and low levels of MHC.

In a classical paper, Jones & Probert (1980) showed that mutant flies with white eyes preferred sites with little light (because more light is uncomfortable to them and they see better in dim light), whereas the lab strain of wild type flies with dark eyes preferred sites with more light (because they see little in dim light). This shows that even inbred, domesticated flies show MHC. They next showed that this behaviour helped to maintain genetic variation in eye colour when flies could choose between these two habitats (variation was lost when there was only one habitat). Nonetheless, the habitat separation between these two types of flies was far from perfect, most likely because not all flies are equally good at monitoring, comparing and moving between habitats in order to make the correct choice (we also see a lot of behavioural variation in student practicals that we are already running for several years now). It is this variation in degree of MHC that we will use to select for populations of flies that are good in making the correct choice given their phenotype, and populations that are less good or even make no choice at all. Heritability for behaviours in general is moderately high (Jaenike & Holt 1991, Charmantier et al. 2014), and there is no reason to believe this isn´t the case here. Nonetheless, we will use outbred populations with greater amounts of genetic variation in order to increase the speed with which the different selection lines will diverge with each generation of selection (Charmantier et al. 2014). Therefore, in a few generations of selection we will have lines which are behaviourally distinct (Fuller et al. 2005).

Selecting for a complex behaviour like MHC might seem somewhat difficult. An effective way to do this is to take a group of offspring that has a similar genetic background (belonging to the same population), but that show variation in the specific phenotype of interest, e.g. because they result from heterotypic matings (say, recessive homozygous white eye * heterozygous wild eye parents, giving 50% white eye and 50% wild type offspring). We then test this entire phenotypically-variable population of flies sequentially multiple times for their habitat choice. To make a high MHC line, the new parents for the next generation are taken from the groups of flies that repeatedly chose the correct habitat, given their phenotype (dark for white eye, bright for wild type). To make a low, non-discriminant MHC line, the new parents are taken from the groups that switched habitats each time habitat choice was tested sequentially (i.e. their choice was independent of their phenotype). By including white-eyed and wild type parents in equal proportions in the new parents, the phenotypic variation is maintained in the selection lines, while free mating keeps them otherwise genetically well-mixed.

We aim to create independent selection lines that show phenotypic variation in one of five different traits (see below). Morover, each line will be replicated to have an independent replicate of the same treatment and to avoid the risk of loosing a line by accident (ref Fuller). This then results in 5 traits * 2 replicates * high/low MHC = 10 high MHC lines and 10 low MHC lines.

TECHNIQUES TO MANIPULATE PHENOTYPES

In the Fruit fly the phenotypic variation needed for habitat choice experiments can be extracted from a range of genetic resources. The first of these are a few classical mutations (see for some examples Figure 5). Pale eyes make individuals more sensitive to light, making them to avoid very bright areas but enabling them to see better in darkness (Jones & Probert 1980). Dark bodies heat up more given a certain amount of light, so mutant dark cindividuals may avoid very bright areas to avoid overheating (Karpestam et al. 2012), whereas pale mutant flies may do the opposite. There are hundreds of phenotypically distinct Fruit fly mutants, and this list will be revised to detect additional candidate mutations that might be relevant for performance in certain environments.

A second resource is the Gal4-UAS system (Duffy 2002) (Figure 6). Gal4 is a yeast transcription activator protein whose production depends on the activation of the promoter to which it has been

linked in a transgenic animal. Some of these promotors are responsive to the environment, e.g. a heat shock, or a certain chemical in the food. Thousands of Gal4 fly strains have been developed in which the production of Gal4 is very tissue-restricted, e.g. only in eyes, or only certain neurons. The Gal4 protein binds with an Upstream Activator Sequence (UAS), which then drives the expression of a gene downstream of the UAS, the effector gene. (This can also be a DNA sequence that produces RNAi, which allows knock-down of a yet another target gene). There are also great numbers of different fly strains with specific effector genes. The exciting methodological aspect of this system is that each Gal4 strain or UAS strain by itself shows little or no phenotypic effects, but by crossing a specific Gal4 strain with a chosen UAS strain, *we can produce specific phenotypic effects in specific tissues by specific environmental stimuli* (Duffy 2002). For example, a mutant strain with white eyes can produce offspring with normal dark eyes after exposing larvae to a heat shock, allowing us to manipulate eye colour in otherwise genetically identical individuals. Or a strain may produce some mildly detrimental substance after eating some food containing a certain chemical, evoking a behavioural response to the environment with that food. There are literally thousands of possible combinations of phenotypic effects, the affected tissues, and the environmental triggers, so that after testing a number of high-potential combinations, we will be able to select several kinds of flies showing relevant phenotypic changes in response to distinct environmental stimuli.

A third resource which shows great promise is the very recent approach of optogenetics (selected by Nature as ´Method of the Year 2010´; http://www.nature.com/nmeth/focus/moy2010/index.html). Here (Figure 7) specific groups of neurons have been made to switch on (or off) due to certain light wave lenghts. For example, flies have been produced that will make a copulatory movement due to a brief pulse of green light, or perform an escape flight after a pulse of blue light. A number of behaviours or physiological states (including those involved in experiencing a pleasant or stressful situation, e.g. Dawydow et al. 2014) can now be activated by exposing the flies to certain colours of light, including colours outside the visual range of the fly (Inagaki et al. 2014), and more developments are expected before the start of the project. We can thereby manipulate the perceived quality of a habitat by simply changing the colour of light pulses, where some individuals would prefer habitats with e.g. red light, whereas others avoid these same habitats just because the light triggers different groups of neurons in otherwise genetically comparable individuals. Because these phenotypes may not be visually distinguishable, additional visual selectively neutral traits will be attached to the utilised physiological/behavioural genotypes.

It is worth pointing out here, that using these techniques poses little logistical problems, as it mainly involves the breeding and crossing of distinct lines of flies (Greenspan 1997, Roberts 1998) (i.e. no molecular work is needed). Dr. Antonio Prado is a specialist in the keeping and breeding of genetically distinct fruit flies. Furthermore, as one of the largest concentrations of researchers working with *Drosophila* in Spain, all these types of flies and the associated techniques are used within our institution, including in the lab of Dr. Casares and Dr. Almudí, who are associated to the proyect and who can provide additional advice and training if necessary. Strains of flies can be easily obtained from scientific stock centres, other researchers, or ordered to be created for us if desired by the Drosophila Transgenesis Centre (Madrid), to whom we are subscribed.

Finally, a last technique to obtain specific Fruit fly phenotypes is the recently developed Crispr Cas9 technique. This method has made it possible to silence specifically selected genes, if the DNA sequence of this gene is known (Sander & Young 2014). This technique involves injecting eggs with a protein and an appropriate guide RNA which attach to the desired sequence and damage the DNA sequences just next to it, thereby disrupting its translation into gene products. Capitalising on the well-annotated *Drosophila* genome and the vast knowledge on biochemical and genetic pathways of a large number of traits, we can create specific mutations which we would like to use. This novel technique is a bit more challenging to use, but it is already in use and has been perfected at my institution, e.g. by working team member Dr. Isabel Almudí. It has high rates of success, is cheap, does not effect non-target genes, and does not involve highly dangerous chemicals.

Overall, in the Fruit fly we therefore can select and test for MHC using individuals whose phenotypes differ because of (i) permanent genetic effects (e.g. the classical white eye colour mutant or new custom-made mutants generated by Crispr), (ii) slow developmental changes with various environmental controls (e.g. white-eyed individuals developing dark eyes after a heat shock), and (iii) instantaneous effects of pulses with light of a certain colour (e.g. a sensation of stress after a pulse of

invisible infra-red light). This broad variation in type and timing of phenotypic change therefore allows us to investigate MHC over many traits, and gives flexibility for habitat manipulation in the experiments.

DETAILS ON THE KEY RESEARCH TOPICS

The research program is an integrative combination of ecological, evolutionary, experimental, physiological, technical and theoretical components. For each research topic we will briefly introduce the proposed research, the rationale for doing so, the chosen methodology, and the reasons for choosing this. (In doing so, we focus on design and methods, and do not discuss details of data analysis when these are standard approaches of proven value, such as Generalised Linear Mixed Models or variations thereof).

(1) Hypothesis: there are physiological drivers of MHC
The general principle behind MHC is that individuals assess their local performance across different habitats (Edelaar et al. 2008). How they do this exactly is difficult to know, but one possibility is that poor performance leads to a range of physiological stress responses which feed back into behavioural decision making. Obtaining more insight into potential physiological responses to exposure to a certain habitat gives us more insight into the drivers of MHC, could confirm that habitat choice is based on a clear and biologically relevant response to habitats, and might provide clues as to how certain fitness consequences are generated. We will assess a number of parameters to judge differential physiological responses (shorter- and longer-term) by a given phenotype to specific habitats:

- Movement rate
- Respiration rate
- Stress hormones
- Oxidative stress levels (damage, enzymatic activity, oxidative capacity)
- Heat-shock protein expression
- Telomere length

Movement rate is easy to score visually or with our infra-red sensors. We have access to micro-chamber respirometry equipment that can be adapted to insects. The other parameters are routinely measured in the Ecological Physiology Laboratory at my second affiliation (EBD-CSIC).

We will measure these parameters in each of the duplicate high MHC lines (10 populations), when exposed to preferred versus non-preferred habitat (20 combinations). For each combination we will measure a number of flies, with a maximum of 20 individuals for the more costly analytical laboratory assays (N = 400 in total).

(2) Hypothesis: MHC influences reproductive rate
MHC means that individuals choose a certain habitat because they evaluate their performance to be better there. In that case, fitness should be higher in the chosen habitat. This is a straightforward prediction that nonetheless should be tested if we are to interpret any observed habitat preference as *matching* habitat choice (Karpestam et al. 2012). A powerful way to test this prediction is to measure the reproductive rate of each phenotype when forced to stay in each habitat, and check if they are indeed fitter in their preferred habitat. In the Fruit fly we can do very simple standard fitness assays by simply counting the number of offspring that come out of vials on which they deposited eggs (Powell 1997). We predict that Fruit flies of a certain phenotype produce more offspring in the habitat that was preferred by flies with that phenotype coming from the high MHC line.

(3) Hypothesis: positive feedback between MHC and adaptation can affect niche shift
MHC might indirectly drive adaptation to certain additional novel aspects that are found only in the chosen habitat, simply because individuals are getting more into contact with this novel aspect then when they would not choose a habitat. They therefore need to deal with this novel aspect even if they choose a certain habitat for other reasons. Interestingly, as a population choosing this habitat becomes adapted to a secondary novel aspect, the adaptation to this novel aspect that individuals now acquired could become an additional reason for them to prefer this habitat (if they now have become even less adapted to the alternative habitat). This way, the evolution of different adaptive traits may exert positive feedback towards an increasing level of MHC, which feeds back into greater adaptation, etc.

Thereby a simple habitat shift in one dimension may transform into a more complex niche shift in various dimensions. This exciting prediction we will test experimentally.

We will use flies from the high MHC lines with two distinct phenotypes, each adapted to a distinct habitat (e.g. eye colour related to illumination), and introduce these flies to a set-up composed of these two habitats. Each habitat has normal food of a limited amount (promoting competition), but we add some vials providing food mixed with a novel, detrimental substance which is different in the two habitats (similar as in Bolnick 2001). These vials provide an initially underutilised, vacant niche aspect in each habitat that individuals could adapt to. We compare the usage of the novel resources by the flies after a few generations of adaptation with the usage by flies that come from two types of controls. Control 1: the same types of flies in the same two habitats that have been kept for the same amount of time but without adding the novel food types. These flies should not adapt to the novel food type, so provide the baseline adaptation to the novel food with which to compare any additional adaptation. Control 2: the same types of flies that have been kept for the same amount of time but in two habitats that do differ in the novel food types but not in the main initial MHC-causing difference between habitats (in this example, illumination). These flies do adapt to the novel food types, but because they do not choose between the two habitats, on average flies are exposed to both novel food types yet only for half the time, so adaptation to each novel food type is compromised and should be slower. This control provides the baseline adaptation to the novel food in the absence of MHC. These treatments will be replicated and continued for 15 generations. When then evaluate success in usage of the novel food types as number of offspring produced on this food type. This tests whether the presence of MHC for one ecological dimension drives adaption in a second dimension. We also evaluate the strength of choice between the two habitats that have the novel food types. This tests if MHC increases because of the adaptation to the new resources. The prediction is that usage of the novel, initially detrimental food types and MHC are both greater in the treatment combining the initial and added environmental differences, because it allowed for positive feedback between MHC and adaptation.

Variations on this theme are possible to increase the success and generality. For example, we could vary the strength of the initial habitat difference in gradual steps across replicates (instead of zero/maximum as now), compare the results when doing the same experiment but with flies from the low MHC lines, or sequentially add subsequent niche dimensions to the treatment (additional detrimental substances or other challenging environmental conditions). A range of chemical substances and environmental conditions that are known to be challenging for fruit flies are available for the experiments (Powell 1997).

(4) Hypothesis: MHC can influence community members
If MHC increases local adaptation and reproductive output, it could increase competitive ability and thereby influence other species (Edelaar et al. 2008, Bolnick et al. 2011). We will therefore test for simple community effects of MHC at two community levels, by adding (i) a competitor (another species of fly) or (ii) a predator (a parasitoid wasp). These competitors and predators we add to each combination of habitat type and Fruit fly phenotype from the high MHC lines: these combinations simulate strong matching (preferred) habitat choice versus strong non-matching (non-preferred) habitat choice. Since competition and predation are often density-dependent, we will vary the density of each of the species involved to increase the probability of finding relevant effects. As a response variable we will count the number of competitor flies or predatory wasps in the next generation. We then test whether there is an effect of habitat type and Fruit fly phenotype on this number, and if the habitat type effect depends on the phenotype (i.e. if there is a statistical interaction between habitat and Fruit fly phenotype). The expectation is that when habitat choice is matching, there will be less competitor flies and a lower proportion of predators per fly. This experimental design will be expanded to compare the results from the same experiments done with flies from the low MHC lines, where we expect that the interactions will be weaker.

Different competitor species of flies and parasitoid wasps are available to find some that work well in our laboratory setting (we already have some previous experience with this). Alternatively or additionally, we could explore the effects on disease transmission (also related to hypothesis 2 on stress and MHC) or micro-organisms competing over food.

(5) Hypothesis: MHC can drive speciation

Speciation is greatly facilitated when traits subject to divergent natural selection also contribute to non-random mating. Such traits have been called 'Magic Traits' (reviewed by Servedio et al. 2011). Matching Habitat Choice has the potential to turn any phenotypic trait into a kind of magic trait, even when the potential for homogenising gene flow is large, because it causes a spatial clustering of individuals with similar phenotypes. If individuals choose the habitats that best match their phenotypes, then phenotypically similar individuals should be found together in the same habitat. If next individuals look for potential mates within this habitat, then MHC should result in positive assortative mating by phenotype, and cause a degree of reproductive isolation between populations with different phenotypes. In contrast to an ordinary magic trait, it is not divergent natural selection, but divergent selection of the environment that causes the reproductive isolation. This straightforward prediction has never been tested experimentally for matching habitat choice (Webster et al. 2012), although we found empirical support for it in our grasshoppers (see above). (Other studies investigating the link between habitat choice and speciation typically depend on genetic preferences for certain habitats as in Rice & Salt 1988, or imprinting on habitats as in Beltman & Metz 2005) – these are fundamentally different phenomena that are not necessarily related to local performance as MHC is, and therefore can lead to radically different predictions (Edelaar & Bolnick 2012, Webster et al. 2012, Fry 2003). A number of experiments will be performed to test the potential of MHC to drive speciation.

(i) If MHC promotes speciation, then this predicts that reproductive isolation between different phenotypes should be greater if MHC is stronger. In order to test this prediction we will assess the degree of reproductive isolation for each of the phenotypic traits in each of the selection lines for high or low MHC, by determining the phenotypes of the offspring generation. We will compare the proportion of mixed matings between assays where flies could choose their own habitat, with the proportion when flies are mated in a single habitat, in order to control for any reproductive isolation between phenotypes unrelated to habitat choice.

(ii) Speciation is hypothesised to be promoted if more traits are simultaneously under selection (Rice & Hostert 1993), but experimental tests of this hypothesis are rare (Nosil 2012). To test the prediction that MHC promotes speciation to a greater extent when it acts on more traits, Fruit flies from the high MHC lines will be used. By making controlled crosses between flies from lines selected for MHC using different traits, we will have access to flies that differ by just one trait or by several traits. Next, these populations varying in one versus several phenotypic traits will be released in an environmental setting that is relevantly heterogeneous for all variable traits. Reproductive isolation (RI) will be measured by scoring the proportion of heterozygous (mixed) offspring from a representative number taken from each of the two habitats. As a control, these populations are also released in an homogeneous environment to assess any reproductive isolation due to assortative mating by phenotype alone. The prediction is that reproductive isolation is greater when more traits are involved in MHC, and greater when MHC is possible. By varying the amount of difference between the environments, we can distinguish between the effects of more traits and the effect of greater total divergent selection (Nosil 2012).

An alternative test of this hypothesis is also possible. We could take flies from the high MHC lines which each vary in only one trait, and introduce flies from these different lines into a setting of two habitats which are opposite in environmental aspects relevant to all traits. The prediction is that over time these different traits should become linked in two genetically diverged populations. These diverged populations should show a greater degree of reproductive isolation (i.e. less hybrid offspring) than that evolved in control experiments, in which we release flies which differ in only one trait (Fry 2003, Nosil 2012). The selection of traits, cage design and cage size will be determined a priori by exhaustive testing of alternatives. As throughout this project, these treatments will be replicated to allow for stronger statistical inference.

(6) Modelling the conditions for and consequences of MHC

Theoretical modelling can greatly generalise and clarify the workings of specific mechanisms. Two aspects of MHC need to be explored in greater detail in order to broaden understanding and acceptance in the scientific community (Bolnick & Otto 2013): (i) under which conditions does it evolve (and when not), and (ii) what are its consequences? Given that MHC basically solves the same

problem of environmental heterogeneity as natural selection and phenotypic plasticity are known to do (see Figure 1), this discussion is best undertaken by a direct comparison with these two alternative mechanisms contributing to higher local performance. The same can be said for comparisons with distinct types of habitat choice, for which the most important ones are genetic habitat preferences (Jaenike & Holt 1991) and imprinting (Davis & Stamps 2004).

Using an individual-based simulation model, we have already made a first exploration of the conditions under which MHC evolves compared to phenotypic plasticity (Edelaar et al. revision in prep. for *American Naturalist*, model adapted from Gomez-Mestre & Jovani (2013). Specifically, we have focused on the degree of environmental change, life history order of development versus dispersal, and the effect of fitness costs for four traits contributing to MHC and plasticity (the potential for change, and the sensitivity to assess habitat variation, for each). Due to its mechanistic flexibility and stochasticity this model provides an excellent basis to develop further insight into which conditions promote or restrain the evolution of these distinct mechanisms, and how mechanisms may act as antagonists or in synergy. We already found strong effects of the costs of each of the four traits contributing to MHC and plasticity, and indications that the degree of kin selection might differ between mechanisms. Likewise we can use this basic model to study the various consequences of MHC for assortative mating, speciation, population dynamics, species-coexistence, invasion of novel habitats, and population and quantitative genetic metrics. These last consequences are already explored in Nicolaus & Edelaar (ready for submission to *Journal of Evolutionary Biology*). We find strong effects of MHC on the maintenance of genetic variation within and between populations, and a strong facilitating effect on reproductive isolation (see Hypothesis 6: speciation). (Dr. Nicolaus is part of the working team). These and other consequences may be investigated in certain ecological contexts, such as: what happens when an invasive species is introduced to a new range, or does MHC promote or constrain adaptation to global change, or what happens if environmental change outpaces the rate of genetic adaptation or developmental plasticity?

Extensive experience with the simulation model is already present, and the approach is ready to be further developed. This will be undertaken with external collaborator Dr. Dries Bonte (see below). Further extensions from a world made of small habitat patches to continuous space and greater population sizes (to reduce the effect of non-adaptive genetic drift and kin competition on the dynamics) may be undertaken with the help of Dr. Maria Carmen Gordillo. She is a molecular physicist also based at the University Pablo de Olavide who applies evolutionary algorithms in her research. She has extensive experience in this modelling approach and is the owner of a computer cluster for mass calculations. Her letter of support is attached to this application (she is not part of the working team due to the new restrictions on participation in more than 1 project, even in the working team). This topic does not have any directly associated costs, apart from some costs for travel to interact with the external collaborators.

TIME SCHEDULE

See Figure 8 for a breakdown of activities and outputs across time as planned. For the listed research topics we indicate when the main work takes place (green) and from which moment papers are expected to be produced (orange). Topics are numbered the same as in the main text. The project will start with the development of the habitat choice protocol, and the testing of different combinations of fly traits and environments (first 6 months), followed by the creation of the selection lines (next 6 months, and thereafter maintained and improved by continued selection for the degree of matching habitat choice). Modelling and conceptual advance will also be started at the beginning. As the selection lines have been created and continue to diverge at the end of the first year, the other topics will be investigated by their respective experiments, avoiding too much overlap among these in time. The number of simultaneous experiments increases gradually, and then decreases again towards the end of the project when there will be more focus on analysis and writing.

RESOURCES AND COLLABORATIONS

The University Pablo de Olavide (http://www.upo.es/portal/impe/web/portada?lang=en) is a small, young university with a strong focus on high-quality research, thereby providing a stimulating and productive research environment. It consistently ranks in the top 3 of most productive (per capita) Spanish universities. The host is committed to provide all necessary space and normal resources for

the successful execution of the project. *It also guarantees the continued employment of the PI in case the proposal is awarded* (signed letter of commitment attached to the application). All people involved in the project at UPO will be supplied with office materials and furniture, and access to all normal institute infrastructure, library and support services. Also not charged to the project will be the use of 2 offices, 1 large laboratory, and the infrastructure we built up in the past years to keep and breed invertebrates (heating mats, specialised lighting).

An additional important resource of techniques and experience present on the UPO campus is the Developmental Biology Institute (CABD; http://www.cabd.es/en-home.html), a mixed centre with CSIC. It houses young and dynamic groups working on *Drosophila*, mouse, Zebra fish, *Xenopus* and *Caenorhabditis* development, and on regulation of gene expression and oxidative stress. Specific to this project, there is a lot of experience on the use and development of transgenic *Drosophila*. We can count on the full cooperation of Dr. Fernando Casares, a specialist in the use and study of transgenic Fruit flies (http://www.cabd.es/en-research_groups-10-26-control-of-organ-growth-and-identity-during-development-and-evolution-lab-members-collaborators.html). He and his lab members have expressed their enthusiasm to provide human capital, training and use of laboratory equipment to the project. Their involvement (Dr. Isabel Almudí is part of the working team) ensures access to experience and techniques with the genetic manipulation of Fruit flies. A letter of support of Dr. Casares is attached to this application (he is not part of the working team due to the new restrictions on participation in more than 1 project, even in the working team).

The Estación Biológica de Doñana (http://www.ebd.csic.es/inicio to which the PI is affiliated, and conveniently also located in Seville) is one of the best places for ecology and evolution in Spain and another great resource for knowledge, experience and analytical techniques, specifically here the techniques to measure metabolic and stress-related parameters. Techniques, feasibility and prices have already been discussed with the laboratory coordinator.

External collaborator Prof. Daniel Bolnick (University of Texas at Austin, USA; https://bolnicklab.wordpress.com/) is a young but already very distinguished, high-profile researcher (see attached CV). He has published extensively, amongst other things, on the ecological and evolutionary drivers of niche width, population divergence, local adaptation, and speciation. He is also very convinced of the importance of matching habitat choice (Bolnick et al. 2009, Bolnick & Otto 2013), and has collaborated in the preparation of the past excellence project involving grasshoppers, as well as in this project involving Fruit flies. Even though he currently mostly works with stickleback fish, he has relevant experience in the design and execution of crucial experiments with Fruit flies (Bolnick 2001). We have co-authored an important paper in which we synthesised various somewhat exotic sources of dispersal under the umbrella of "non-random gene flow", an evolutionary phenomenon that didn´t have its own term and that had been relatively neglected (Edelaar & Bolnick 2012). I have visited Dr. Bolnick for two weeks in November 2015 in order to advance on the publication of the main results of the research on grasshoppers of part of the past project. This has been very fruitful, and we believe we have come up with a novel conceptual framework for the mechanisms of adaptation that will be very helpful to the scientific community and that should catalyse future studies (Fig. 1). Because of his involvement in the project design and writing, and data analysis and interpretation, he will be a co-author on this paper (aimed for Nature). Also for this current project we plan continued interaction, including discussion of plans, experimental designs and results, mutual visits, theoretical concept and model development, and exchange of lab members. If possible we will also co-organise a workshop in which we bring together biologists and mathematicians in order to integrate the process of matching habitat choice into current mathematical evolutionary theory.

External collaborator Prof. Dries Bonte (University of Ghent, Belgium; http://www.ecology.ugent.be/terec/research.php?page=see) is an internationally recognised expert in the field of movement ecology (see attached CV). Working on many species and in many habitats, his group integrates ecological and evolutionary concepts into a spatial perspective, and they have published extensively on this. They aim to understand how the spatial configuration and diversity of habitats affect ecological dynamics, by scaling up from individual responses to macroscopic patterns like the emergence of food webs and range sizes. As part of this research he is modelling the interaction between dispersal and local adaptation, and he has started to include the operation of matching habitat choice into his models. We therefore decided to join our insights and to interact and

collaborate on the further development of the models, based on both his and our previous simulation models. I will visit him and his group from 5-7 of June 2016 to present my previous and ongoing work. This visit will already present a start of the collaboration, before the beginning of this proposed project. As his group also works with laboratory populations of an invertebrate (a spider mite) selected for dispersal behaviour, he is also in the excellent position to provide feedback on the experimental design and interpretation. If financially possible, we will organise the exchange of group members.

REQUESTED HUMAN RESOURCES

We ask for a full-time technician. This person is needed to help out with the time-consuming creation and maintenance of all fly lines. Furthermore, he/she will assist with the logistics and execution of the various experiments. The technician will also be responsible for the everyday running of the lab (purchase, maintenance and cleaning of materials), and the practical instruction and ongoing coordination of students. These various tasks are too important to leave them up to more temporary people like master students, and too time-consuming to be taken care of by the members of the scientific team alone.

C.2. IMPACTO ESPERADO DE LOS RESULTADOS

Scientific impact

Matching Habitat Choice (MHC) can have impacts on a broad range of phenomena which are key topics in the fields of ecology, evolution and conservation biology, such as the rate and degree of local adaptation, the carrying capacity and stability of populations, the maintenance of genetic variation, the genetic divergence of populations, the evolution of ecological niches, the rate and success of adapting to or invading novel habitats, and the probability of speciation (Edelaar & Bolnick 2012, -edelaar et al. 2008, Bolnick & Otto 2013). Despite these varied and important potential impacts, only a handful of theoretical and empirical papers have dealt with this topic directly (reviewed in Edelaar et al. 2008, Ravigné et al. 2009, Bolnick & Otto 2013, Karpestam et al. 2012), with other papers giving indirect evidence or only passing attention.

Given this background, we anticipate the following impact. (1) The research proposed here addresses a knowledge-gap (the effects and importance of MHC) in a big question in ecology and evolution (the process of adaptation), and is therefore timely. Invitations to write papers on this topic (Ecology Letters, Trends in Ecology & Evolution), and to edit a book (Springer) and a special issue (Evolutionary Ecology) support this claim. While we have published an integrative paper on "non-random gene flow" (which includes MHC) in Trends in Ecology & Evolution (Edelaar & Bolnick 2012), and have edited a special issue on non-random and asymmetric gene flow for Evolutionary Ecology (November 2015), there is still much to be discovered. We will test many predictions and topics never tested before in this context. Obtaining first results and ´proofs-of-principle´ on the proposed research topics (both empirical and theoretical) will thus enable us to convince the scientific community of the workings and especially the relevance of MHC. (2) Since insects are relatively simple organisms, any positive results will challenge other researchers to consider whether MHC is important for their more complex system, especially for more mobile and cognitive organisms. (3) Working with the Fruit fly model, which is easily kept and replicated or shared, allows other researcher to profitably expand upon our research, thus enhancing the impact of our findings (Powell 1997). (4) We will, as far as we know for the first time, employ a number of modern techniques and resources from developmental biology and neurobiology (Sander & Young 2014, Duffy 2002, Inagaki et al. 2014) in a completely different, eco-evolutionary context. This may stimulate others to do the same in these or unrelated research topics.

Overall, we aim to refine the way people think about evolution, adaptation and dispersal, for MHC to become a standard potential explanation for a variety of observed evolutionary patterns, for people to apply the MHC concept to their own work (including conservation), and thereby for our research to act as a catalyst for future studies within related and even unrelated (incl. applied) fields. Having such a richer and more complete understanding of adaptation and evolution can only be a benefit to us all (Losos et al. 2013).

Transmitting results

It is foreseen that several key, eye-opening results can be published in major international peer-reviewed specialist and perhaps even multidisciplinary journals. We ask for funding that enables us to publish the results as Open Access articles. These publications will be highlighted to the press and the public through press releases prepared in collaboration with the responsible offices of the University Pablo de Olavide and the collaborating institutions. In our experience these press releases result in interviews with radio, TV and the written press, and are also quickly picked up by many web sites and bloggers across the world, all enhancing public exposure. We will also highlight our results through our own web sites, blogs and Twitter accounts. Additional diffusion to the scientific community will be achieved by presenting talks and posters at international conferences, and by invited seminars.

C.3. CAPACIDAD FORMATIVA DEL EQUIPO SOLICITANTE

The group of applicants includes a relatively young PI who is successfully establishing a new research line. Given the width and modular nature of the proposed research, there is excellent scope to produce an attractive and high-quality thesis by a PhD student. This student will be supervised by Dr. Edelaar, and co-supervised by Dr. Prado, Dr. Bonte and/or Dr. Bolnick, depending on personal interests and development. The contributions of the PhD student would significantly improve the execution and outcomes of the project, and probably would allow for a reduction of the technician to a part-time contract. The student will take a main role in the experiments that are testing for the ecological and evolutionary consequences of Matching Habitat Choice, so we will be looking for someone with the capacities and motivation to work with live flies and with prior training in the area of evolutionary ecology. This person will receive further training in the design and management of experiments, the collection of behavioural data at the individual and population level, theory in ecology and evolution, modern statistical analysis of biological data, and the scientific and popular reporting of research results in written and spoken form for the scientific community and the general public.

The student will be based at the University Pablo de Olavide. This young and growing university is consistently ranked in the top-3 nationally in terms of per-capita scientific productivity. He/she will participate in courses and other formative activities of the doctoral program "*Environmental Sciences and Society*", of which Dr. Edelaar is member of the Academic Committee, as well as external courses, workshops and meetings.

The research team already has considerable experience in hosting and training of young scientists, as listed here.

Post-doctoral fellows

- Dr. Marion Nicolaus (Postdoctoral fellowship by Subprograma Estatal de Incorporación, 2015-2016). "*Modelling the Consequences of Matching Habitat Choice*". With Pim Edelaar.

- Dr. Alejandro Centenos Cuadros (Postdoctoral fellowship by Programa de Fortalecimiento, Plan Propio de la Universidad Pablo de Olavide, 2015-2016). "*Selection on Genes of the Major Histocompatibility Complex during Biological Invasion*". With Pim Edelaar.

Doctoral theses defended

- Dr. Gloria del Valle. "*Serotonina y aprendizaje instrumental*". Universidad Pablo de Olavide (2003). Supervisors: José María Delgado García & Antonio Prado Moreno.

Gloria is now full professor at the Faculty of Natural and Exact Sciences, National University of Catamarca, Argentina.

Doctoral theses in progress

- Adrian Baños Villalba. "*Neglected Mechanisms of Adaptation to a Changing World*". Universidad Pablo de Olavide. Supervisors: Pim Edelaar & José L. Tella (EBD-CSIC) (planned defence Feb. 2018; 2 papers published so far, 1 submitted, 2 in preparation).

- Juan Ramón Peralta Rincón "*Causes and Consequences of Matching Habitat Choice: an Experimental Approach*". Universidad Pablo de Olavide. Supervisor: Pim Edelaar (a very motivated and promising student. Unfortunately just starting and still without funding, he is gaining experience with Fruit flies in order to collect data on their behaviour and habitat choice).

Master theses defended (last 10 years)

- Tianhong Gong (MSc Biology - Uppsala University, Sweden) "*Signatures of natural selection on crossbills by comparing morphology and genetic markers across populations and age cohorts*" (Pim Edelaar)

- Kerstin Engelmann (MSc Biology – University of Würzberg, Germany) "*Behavioural effects of electromagnetic fields on* Drosophila melanogaster" (Antonio Prado)

- Clara Alcántara-Dominguez (MSc Biodiversity and Conservation Biology – University Pablo de Olavide) "*Making the best of a bad job? Brown plumage when red cannot be produced in weavers*" (Pim Edelaar)

- Alvaro Luna (MSc Biodiversity and Conservation Biology – University Pablo de Olavide) "*Getting to know our new neighbour: adaptation of reproductive timing and social perception of the invasive Rose-ringed parakeet*" (Pim Edelaar)

- David Pablo Queveda Colmena (MSc Biodiversity and Conservation Biology – University Pablo de Olavide) "*Testing for genetic and environmental effects on habitat choice as a mechanism to enhance local crypsis in a grasshopper*" (Pim Edelaar)

- *Aída Jordán Andrade* (MSc Biodiversity and Conservation Biology – Universidad Pablo de Olavide) "*Experimental tests of Matching Habitat Choice*" (Pim Edelaar)

- *Francisco Javier García de Andoín* (MSc Applied Ethology and Animal Behaviour – Universidad Pablo de Olavide) "*Behavioural and physiological responses to variable environments*" (Pim Edelaar)

- Paola Micaela Ozzano (MSc Neuroscience and Behavioural Biology – University Pablo de Olavide) "*Caracterización genética y celular de una población de células troncales en* Drosophila*: los neuroblastos larvales*" (Antonio Prado, tutor)

- Mariam Daniels Rodríguez (MSc Neuroscience and Behavioural Biology – University Pablo de Olavide) "*Eficacia en la utilización de un sistema de rastreo visual (eye tracking) para evaluar los procesos de atención visual en una muestra de niños con déficit de atención con hiperactividad (TDAH) en comparación con un grupo control*" (Antonio Prado, tutor)

- Yohami Fernández Delgado (MSc Neuroscience and Behavioural Biology – University Pablo de Olavide) "*Comunicación acústica intraespecífica en* Empyreuma pugione *(Erebidae, Arctiinae)*" (Antonio Prado, tutor)

Direction of other undergraduate student projects *("trabajos fin de grado, alumnos internos, tesinas escuelas técnicas"):*

- more than 25 students

External member of PhD Advisory Committee

- *Yimen Araya,* International Max Planck Research School for Organismal Biology*,* Dept. of Behavioural Ecology and Evolutionary Genetics, Max Planck Institute for Ornithology, Seewiesen, Germany (defended 6 July 2015) (Pim Edelaar)

Host of **Leonardo da Vinci** ***visiting post-graduate***

- Rowan Doff, Sept. 2013-Dec. 2013 (EU-funded) (Pim Edelaar)

Post-graduate teaching

Dr. Edelaar is the coordinator of the undergraduate course "Conservation Genetics", part of the fourth year of the bachelor study *Environmental Sciences* (University Pablo de Olavide). He also is a lecturer in one module of the MSc program *Biodiversity and Conservation Biology*, and in two modules of the MSc program *Applied Ethology and Animal Behaviour* (both University Pablo de Olavide). Furthermore, he has developed an introductory mini-course on the use of three statistical approaches: classical frequentist null hypothesis testing using p-values, information-based multi-model comparison using various Information Criteria, and model fitting on Bayesian principles yielding posterior distributions of parameters of interest. He is also member of the Academic Committee of the doctoral program "*Environmental Sciences and Society*", and in that role has stimulated, developed and organised courses for PhD students on writing scientific papers, analysis and simulation of data using the program R, the use of GIS in research, and bibliographic research.

Dr. Prado has repeatedly taught on 3 different PhD courses: "Genetics and Molecular Biology", "Experimental Analysis in Biology", and "Neuroscience and Behavioural Biology". He also taught on a national course on neuroscience. He is furthermore lecturer in the MSc program "*Applied Ethology and Animal Behaviour*" and for teachers for secondary schools (University Pablo de Olavide).

Additional training and exposure

Besides the training at the University Pablo de Olavide, the student should be able to interact with people and other students at the Estación Biológica de Doñana (CSIC, with which Dr. Edelaar is affiliated) via visits, seminars and courses. We also will plan extended training stays and the participation in courses at the institutes of our collaborators in Belgium (Dr. Dries Bonte, University of Ghent) and the USA (Dr. Dan Bolnick, University of Texas), which together have directed 17 PhD theses. Depending on development and affinities, these interactions might take the shape of co-direction of the thesis as well.

C.4. IMPLICACIONES ÉTICAS Y/O DE BIOSEGURIDAD

Fruit flies will only be used and bred in the laboratory, not released in the field. Wild flies will be collected in order to produce outbred, genetically more variable strains. Wild flies are common and not protected. For this insect species no ethical review is necessary as far as we have been informed, so we do not provide further information on procedures, experimental design, numbers used etc.

Some of the fly strains we plan to use are transgenic. Genes from yeast and certain algae have been introduced using P elements and the vector φC31. Such flies are part of the well-known GAL4-UAS system, and the more recent technique of optogenetics. The aim of the manipulations has been to control the expression of certain genes in certain tissues and specific neurons. These flies will be used only in captive conditions within a closed laboratory, with no releases into the wild. Accidental escapes within the lab will be eliminated by placing sufficient fly traps. At the end of experiments,

flies will be sacrificed by anaesthesia with CO_2 followed by emersion in a soapy mixture of alcohol and water. All these procedures follow common practice in other labs working with these types of flies (Roberts 1998).

If these experiments go ahead, any necessary official local, regional and national ethical permissions will be sought, and if necessary experiments will be monitored by the relevant authorities.

References

Beltman, Metz 2005. Speciation: more likely through a genetic or through a learned habitat preference? Proc R Soc Lond 272:1455-1463

Bolnick 2001. Intraspecific competition favours niche width expansion in *Drosophila melanogaster*. Nature 410:463-466

Bolnick, Amarasekare, Áraujo, Bürger, Levine, Novak, Rudolf, Schreiber, Urban, Vasseur 2011. Why intraspecific trait variation matters in community ecology. Trends Ecol Evol 26:183-192

Bolnick, Otto 2013. The magnitude of local adaptation under genotype-dependent dispersal. Ecol Evol 3:4722-4735

Bolnick, Snowberg, Patenia, Lau, Stutz, Ingram 2009. Phenotype-dependent native habitat preference facilitates divergence between parapatric lake and stream stickleback. Evolution 63:2004-2016

Charmantier, Garant, Kruuk 2014. Quantitative Genetics in the Wild. Oxford University Press

Davis, Stamps 2004. The effect of natal experience on habitat preferences. Trends Ecol Evol 19:411–416

Dawydow et al. 2014. Channelrhodopsin-2-XXL, a powerful optogenetic tool for low-light applications. PNAS 111:13972-13977

Duffy 2002. GAL4 system in *Drosophila*: a fly geneticist's Swiss army knife. Genesis 34:1-15

Edelaar & Bolnick 2012. Non-random gene flow: an underappreciated force in ecology and evolution. Trends Ecol Evol 27:659-665

Edelaar, Alonso, Lagerveld, Senar & Björklund 2012. Population differentiation and restricted gene flow in Spanish crossbills: not isolation-by-distance but isolation-by-ecology. J Evol Biol 25:417-430

Edelaar, Siepielski, Clobert 2008. Matching habitat choice causes directed gene flow: a neglected dimension in evolution and ecology. Evolution 62:2462-2472

Fry 2003. Multilocus models of sympatric speciation: Bush vs. Rice vs. Felsenstein. Evolution 57:1735–1746

Fuller, Baer, Travis 2005. How and when selection experiments might actually be useful. Integr & Comp Biol 45:391-404

Gomez-Mestre, Jovani 2013. A heuristic model on the role of plasticity in evolution: plasticity increases adaptation, population viability, and genetic variation. Proc R Soc B 280:20131869

Greenspan 1997. Fly Pushing – The Theory and Practice of *Drosophila* Genetics. Cold Spring Habor Lab Press

Holt, Barfield 2008. Habitat selection and niche conservatism. Israel J Ecol Evol 54:295-309

Inagaki et al. 2014. Optogenetic control of *Drosophila* using a red-shifted channelrhodopsin reveals experience-dependent influences on courtship. Nature Methods 11:325-332

Jaenike, Holt 1991. Genetic variation for habitat preference: evidence and explanations. Am Nat 137:S67–S90

Jones, Probert 1980. Habitat selection maintains a deleterious allele in a heterogeneus environment. Nature 287:632-633

Karpestam, Wennersten, Forsman 2012. Matching habitat choice by experimentally mismatched phenotypes. Evol Ecol 26:893-907

Kawecki, Ebert 2004. Conceptual issues in local adaptation. Ecol Lett 7:1225–1241

Laland et al. versus Wray et al. 2014. Does evolutionary theory need a rethink? Nature 514:161-164

Losos et al. 2013 Evolutionary biology for the 21st century. PloS Biol 11:e1001466

Nosil 2012. Ecological Speciation. Oxford University Press

Powell 1997. Progress and Prospects in Evolutionary Biology – the *Drosophila* Model. Oxford University Press

Ravigné, Dieckmann, Olivieri 2009. Live where you thrive: joint evolution of habitat choice and local adaptation facilitates specialization and promotes diversity. Am Nat 174:E141-E169

Ravigné, Olivieri, Dieckmann 2004. Implications of habitat choice for protected polymorphisms. Evol Ecol Res 6:125–145

Rice, Hostert 1993. Laboratory experiments on speciation: what have we learned in 40 years? Evolution 47:1637–1653

Rice, Salt 1988. Speciation via disruptive selection on habitat preference: experimental evidence. Am Nat 131:911-917

Roberts 1998. *Drosophila* – a Practical Approach. Oxford University Press

Sander & Young 2014. CRISPR-Cas systems for editing, regulating and targeting genomes. Nature Biotech 32:347-355

Servedio, Van Doorn, Kopp, Frame, Nosil 2011. Magic traits in speciation: 'magic' but not rare? Trends Ecol Evol 26:389–397

Webster, Galindo, Grahame, Butlin 2012. Habitat choice and speciation. Int J Ecol:154686.