INTRODUCTION

Epistasis is the phenotypic effect of non-linear interactions among genes. The polygenic nature of quantitative traits, where gene products are part of metabolic or developmental networks, ensures that the effect of one allele depends on allelic variants at other loci (1, 10). In a population this “physiological” epistasis, which reveals the interaction between molecules, is only seen when allelic variation is present at interacting loci. Epistatic effects are statistically defined as the deviation of multi-locus genetic values from the additive combination of their single locus components (15), and depend on “physiological” epistasis and also on allele frequencies. Since the average effect of substituting one allele in a population is a function of alleles in interacting loci, epistatic effects can contribute also to additive genetic variance (see Eq. in annexed file A1 for phenotypic variances components) in a frequency-dependent manner (16, see also Eq. 2 in file A1).

Epistasis in evolution

The role of epistasis in evolution and more specifically on the adaptation of natural populations has been subject to a longstanding debate (2). Many situations exist where epistasis is likely to be of significance (1). For example, epistasis can influence the rates of adaptation to new environments (3). Since only the additive component of genetic variance, which determines the heritable proportion of phenotypic variability (15) matters for response to natural selection (Eq. 3 in file A1), high levels of epistatic variance may reduce the rate of adaptation. When allele fixations occur, as a result of drift after population bottlenecks or in subdivided populations (17), or in response to selection (18), the contribution of epistatic effects to additive variance increases, promoting further adaptation.

Epistasis may also constrain the selective accessibility of trajectories to high fitness genotypes, in the presence of several well-fit allele combinations without favourable intermediates (4). This implies the existence of multiple fitness peaks and fitness valleys in rugged landscapes (10) that favour local adaptation of populations. Adaptation in such a landscape may lead to selection of alternative allele combinations in different populations by chance, creating several “co-adapted gene complexes”. Hybridization of such locally adapted populations may be impossible since deleterious, multi-locus alleles would be formed by recombination, as proposed in the Dobzhansky-Muller model for speciation (5) (see Fig.1 in annexed file A2).

The non-linearity of phenotypic expression brought by epistasis can also act as a "buffering" mechanism relevant in robustness (7) and in the maintenance of polymorphism by reducing the effects of segregation of deleterious alleles (20). This may be important for the explanation of the evolution of sex and recombination. The appearance and maintenance of sexual recombination may have been selected as a way of purging deleterious genotypes, as the breaking up of allele associations by recombination allows the creation of new combinations free of such mutations (6).

Empirical data

Many studies, which include the comparison of phenotypes of single and double-mutants (20), quantitative trait loci (QTL) mapping experiments (21) or association studies (22) have now been able to show the pervasiveness of epistatic interactions, in several model organisms (8, 21, 23) and in humans (22). Epistatic effects were often found to be of the same magnitude of additive effects and even involve multiple interactions, as for hybrid sterility of *Drosophila pseudoobscura* populations (24). Despite this, a major role for epistasis in evolution remains to be shown (9), probably due to a difficulty in translating epistatic "physiological" effects into population variances (Eq. 1 in file A1). Associating epistatic effects only with the epistatic component of genetic variance or underestimating epistatic variance by maximizing the variation of additive terms in regression analysis (15) limits the relevance of many studies for evolutionary purposes (10).

Experimental evolution

Experimental evolution studies evaluate directly the dynamics of genetic systems and have already contributed to the study of epistasis in evolution (25). Population size, selection environment and recombination can be manipulated and their effects on phenotypic traits recorded. Allele frequencies and fitness proxies can also be tracked over time. Availability of genotypic or phenotypic trajectories, parallel analysis of selection and greater statistical power by the inclusion of replicates are advantages of experimental evolution studies. The development of multi-locus associations, at the DNA sequence level, and their fitness consequences, which can be quantified across generations and populations is particularly helpful for the study of epistasis (14).

Although different experimental systems have, for instance, revealed the importance of epistasis for adaptation in viruses (23), its dynamic nature in *Escherichia coli* (26) or its preponderant source of additive genetic variance in *Chlamydomonas*

reinhardtii (27), the lack of data regarding natural selection on higher organisms is surprising.

By analyzing changes in the genotype-phenotype map in the evolution of outbred, lab-adapted *Drosophila melanogaster* populations, this project will provide a bridge between functional analysis of epistasis that characterizes molecular genetics and the impact of epistasis on adaptation that has characterized much of the debate in evolutionary biology. In a well-known evolutionary experimental system we specifically aim to:

1. Describe allelic variants trajectories in equilibrium and during the process of adaptation;
2. Estimate drift and selection effects on allele frequency dynamics;
3. Estimate the contribution of epistatic effects to additive genetic variances;
4. Determine the evolution of linkage disequilibrium as a result of epistasis and recombination.