Aplicações evolutivas (2021/2022)

O projeto de investigação

Tarefas

O projeto de investigação

Designação da tarefa Task denomination	**Data de início** Start date	**Data de fim** End date	**Duração** Duration	**Pessoas * mês** Person * months
Discovery of polymorphisms at specific loci	01-09-2009	30-06-2010	10	11

Descrição da tarefa e Resultados Esperados
Task description and Expected results

In a first stage a SNP survey needs to be done. For this purpose, genomic DNA of a control population will be sequenced at the loci under study. We expect this to provide information about neutral or nearly-neutral variants in the control environment (we may also find polymorphisms that are being kept by balancing selection). Common lab practice in our group indicates that PCR amplification of DNA extracted from a pool of individuals followed by bulk sequencing of cloned amplicons provides the most efficient way of polymorphism detection for several loci at the same time. Two different regions will be sequenced for each locus, increasing the available information as a consequence of having generally less correlation among SNPs.

Fig. 4 (annexed file A2) shows the location of random loci in the 3L chromosome. The following loci, related to glicolysis will also be analysed: ugp (CG4347), tps1 (CG4104) treh (CG9364), hex-A and hex-C (CG3001, CG8094), pgi (CG8251), pfk (CG4001), ald (CG6058), tpi (CG2171), pgk (CG3127), pglym78 (CG1721), eno (CG17654), pky (CG7070), gpdh (CG90442), pgd (CG3724), glyS (CG6904) and glyP (CG7254) (see also Fig. 6 in annexed file A2, for pathway description).

For each of these 30 loci:

- Primers will be designed for the PCR amplification of two 700 bp regions that are 1-2 kb apart;
- PCR amplification will be done with Expand High-FidelityPLUS Taq polymerase in a 96 well thermal cycler. Template genomic DNA comes from a pool of 20 female flies from a control population (an IB population at generation 690) and has already been extracted and tested;
- Purified PCR products will be ligated to pGEMT-easy vector and cloned in a E. coli (MRF') strain;
- Sequencing of 60 clones (30 x 2 amplicons) will be done with Applied Biosystems reagents and run in an ABI 3130XL sequencer available at IGC. By having 30 clones sequenced, the probability of missing a variant that segregates with a minimum-allele frequency (MAF) of 0.05 will be 0.21 and for an allele with MAF = 0.1 will be bellow 0.05.

We expect to obtain good quality sequences allowing the detection of polymorphisms and a description of diversity for at least 23 loci. If we don't get enough SNP information for those required 23 loci (may be because a region might be under strong negative selection pressures and allele variants segregate at very low frequencies) a population subjected to one of the diversifying environments (like a "CO" population) can also be sequenced in order to increase the power to detect SNPs. We expect this to work specially in cases where the selection pressures are specific to the ancestral environment.

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Tarefa – descrição detalhada do trabalho experimental

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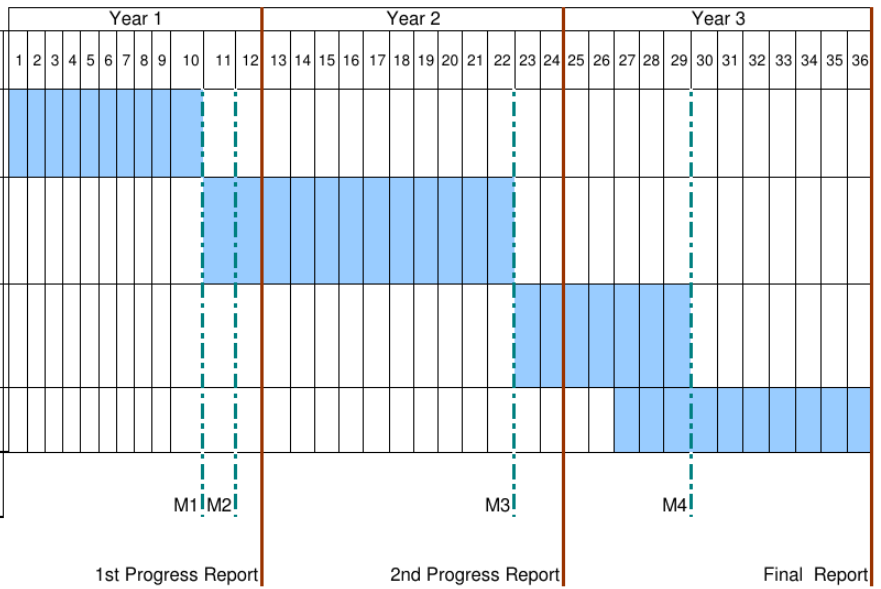
Cronograma – Visualização das tarefas no tempo de execução

Project reference : PTDC/BIA-BEC/099028/2008

Project title: Analysis of epistatic effects during adaptation of *Drosophila melanogaster* experimental populations

Task Nº	Task Denomination	Person*month
1	Discovery of polymorphisms at specific loci	11,00
2	Genotyping of polymorphisms in control, selected and reverse-evolved populations	13,20
3	Data analysis	7,70
4	Simulation studies	10,30
		42,20



Tarefa

Escrever tarefas

→ **Título**

→ **Tempo de realização previsto** (“mês *x* a mês *y* do projeto)

→ **1 frase com o objetivo da Tarefa**

Fazer cronograma

→ **Nº tarefa**

→ **Nome da tarefa**

→ **Tempo em execução previstos, durante o projeto**