2. Der Dissertation zugrunde liegende Publikationen und Manuskripte

2.1 Fine scale mapping in the sex locus region of the honey bee

*(Apis mellifera)*

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**Abstract**

Isolating an unknown gene with fine-scale mapping is possible in a ‘non-model’ organism. Sex determination in honey bees consists of a single locus (sex locus) with several complementary alleles. Diploid females are heterozygous at the sex locus, whereas haploid males arise from unfertilized eggs and are hemizygous. The construction of specific inbred crosses facilitates fine scale mapping in the sex locus region of the honey bee. The high recombination rate in the honey bee reduces the physical distance between markers compared with model organisms and facilitates a novel gene isolation strategy based on step-wise creation of new markers within small physical distances. We show that distances less than 25 kb can be efficiently mapped with a mapping population of only 1000 individuals. The procedure described here will accelerate the mapping, analysis and isolation of honey bee genes.
2.2. The Gene csd is the Primary Signal for Sexual Development in the Honeybee and Encodes an SR-Type Protein

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**Summary**

Haplodiploid organisms comprise about 20\% of animals. Males develop from unfertilized eggs while females are derived from fertilized eggs. The underlying mechanisms of sex determination, however, appear to be diverse and are poorly understood. We have dissected the \textit{complementary sex determiner} (csd) locus in the honeybee to understand its molecular basis. In this species, csd acts as the primary sex-determining signal with several alleles segregating in populations. Males are hemizygous and females are heterozygous at this locus; nonreproducing diploid males occur when the locus is homozygous. We have characterized csd by positional cloning and repression analysis. csd alleles are highly variable and no transcription differences were found between sexes. These results establish csd as a primary signal that governs sexual development by its allelic composition. Structural similarity of csd with \textit{tra} genes of Dipteran insects suggests some functional relation of what would otherwise appear to be unrelated sex-determination mechanisms.
2.3. Signatures of selection among sex-determining alleles of the honey bee

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Abstract
Patterns of DNA polymorphism in genes are a primary tool to dissect signatures of selection, however, the underlying selective forces are poorly understood in most cases. Theory predicts that diversifying selection operates on the primary gene of sexual regulation in the honey bee, the complementary sex determiner (Beye et al. 2003 Cell, 114, 419-429). Females are derived from fertilized eggs and have two allelic versions of csd. Males result when there is only one version of specificity in either the diploid (fertilized) or haploid (unfertilized) eggs. The diploid homozygous males, however, don’t survive which implies a strong diversifying selection regime. The first cDNA csd sequences provide us with the unique power to dissect these signatures that occur during the course of evolution. Large differences in csd sequences within and between four populations were found. They fall into major groups, haplotype I and II. Within haplotype I several allelic lineages were identified. Long resistance time and an excess of nonsynonymous over synonymous differences suggest that diversifying selection has operated on very confined parts of the protein. Signatures of hitchhiking indicate some functional significance of a hypervariable NY repeat region. The first csd sequence data are a notable basis to improve bee selection programs by allele assisted breeding. The results have essential implications to understand the function and evolution of the multi-allelic mechanism that is found in many hymenopteran species (ants, wasps, bees, sawflies). Thus, csd provides an elegant system to study adaptive processes directly on the level of nucleotide sequences.
2.4. Signatures of intra- and genomic recombination on a locus under strong balancing selection

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Manuskript für: Genetics

Abstract
The gene complementary sex determiner (csd) in the honey bee is under strong overdominant selection in which homozygotes have zero fitness, heterozygotes develop into females and hemizygotes differentiate into males. To examine the impact of the overdominant locus at linked genomic regions, nucleotide diversity was measured in different distances to csd. We found that nucleotide diversity was strongly increased with lowering the distance to csd. However, the influence of balancing selection on linked nucleotide polymorphism rapidly decreases due to recombination. Recombination rate in the csd advanced genomic region increases within 43 kb which suggests that recombination has been positively selected to uncouple csd-alleles from the rest of its linkage group. Furthermore, we examined if intragenic recombination occurs in csd contributing to the observed nucleotide pattern in csd-alleles. Our results suggest that recombination had a major role in shaping nucleotide differences of the sex-determining locus.
2.5. Specific developmental gene silencing in the honey bee using a homeobox motif

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**Abstract**

Manipulating the expression of genes in species that are not currently used as genetic models will provide comparative insights into the evolution of gene functions. However the experimental tools in doing so are limited in species that have not served as models for genetic studies. We have examined the effects of double stranded RNA (dsRNA) in the honey bee, an insect with considerably basic scientific interest. dsRNA derived from a 300 bp stretch of the E30 homeobox motif was injected into honey bee embryos at the anterior pole in the preblastoderm stage. We found that the dsRNA fragment successfully disrupted the protein expression of the target gene throughout the whole embryo. The disruption caused deficient phenotypes similar to known loss of function mutants of Drosophila engrailed, whereas embryos injected with nonsense dsRNA showed no abnormalities. We show that the large size of the honey bee egg (D: 0.3 mm, L: 1.6 mm) and the long preblastoderm stage (11–12 h) can be exploited to generate embryos with partial disruption of gene function, which may provide an elegant alternative to classical chimeric analyses. This is the first report of targeted disruption of gene function in the honey bee, and the results prove that the chosen target gene is a functional ortholog to engrailed in Drosophila.