Resources For Comparative Linkage Mapping In Lepidoptera.

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We are developing tools for comparative linkage mapping in Lepidoptera. Specifically, we want to assess the extent of synteny, or gene linkage, and microsynteny, or fine structure, in lepidopteran chromosomes, information that can facilitate gene tracking, positional cloning, finding transgene landing sites, population and ecological studies, and phylogenetic reconstruction. Lepidopteran chromosomes are holocentric, with many microtubule attachment points, in contrast to those of most Metazoa, which are monocentric. This distinctive architecture suggests the possibility that chromosome fragments might persist through many cell divisions, leading to a high level of karyotypic change by chromosome fragmentation or rearrangement. This, in turn, may have contributed to the explosive radiation of lepidopteran lineages (>170,000 extant species today) that occurred between 50 and 100 million years ago. As a reference for comparative studies, we have expanded established genetic linkage maps (Nagaraju & Goldsmith, 2002) for the domesticated silkworm, *Bombyx mori*, to >100 well-conserved codominant molecular markers, based on RFLPs, cloned RAPD sequences, and newly mapped cDNAs and STSs from highly conserved cDNAs in SilkBase (www.ab.a.u-tokyo.ac.jp/silkbase) and other public databases. We have also begun testing the feasibility of using two-color chromosomal Fluorescence In Situ Hybridization to assess linkage in species that are not readily used for inheritance studies. We detected the large rDNA cluster (~240 copies) on chromosome 11 in 4th–5th instar larval testes spreads with ~2 kb plasmids, but saw few reliable signals with cDNAs up to 5 kb using indirect labeling systems (biotin:avidin-Texas red-anti-avidin and digoxygenin:FITC-anti-dig). Larger genomic fragments from BAC libraries (~150 kb) and use of larval ovarian tissue for chromosome spreads yielded more consistent results, and we are now optimizing protocols using model chromosomes with linked markers whose recombination distances are unknown. To serve as chromosomal landmarks and as a source of additional markers to examine detailed gene organization and microsynteny, we have constructed contigs covering >30 genetically mapped ESTs by DNA fingerprinting using two new silkworm BAC libraries (>10X coverage using *Eco* RI and *Hind* III partial digests with ave. insert size of 151 and 166 kb, respectively). For use in initial comparative studies we have also constructed *Eco* RI and *Bam* HI BAC libraries with 10.7X coverage for the tobacco hornworm, *Manduca sexta*, which is in the same superfamily as *B. mori* (Bombycoidea), and 13X coverage for *Heliothis virescens*, which is in a different superfamily (Noctuoidea). Average insert sizes for these libraries are 150 kb, 165 kb, 145 kb, and 145 kb, respectively. Comparisons will be facilitated by similar chromosome numbers (N=28, 28, and 31 for the silkworm, the hornworm, and the budworm, respectively) and genome sizes (~530 Mb *vs* 500 Mb, for *Bombyx vs Manduca* and *Heliothis*), and high levels of sequence conservation in protein-coding genes will aid identification of orthologous genes.