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COMPARATIVE GENETIC LINKAGE MAPPING IN INSECTS

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INTRODUCTION

The Scope of Comparative Genome Organization

Genomics is the study of the structure and organization of entire genomes. The recent coinage of this term (46) reflects the comparative youth of this emerging field. The nuclear genome of eukaryotic organisms is the collection of all DNA sequences in the nucleus that are transmitted through cell lineages by the orthodox processes of mitosis and meiosis. Eukaryotic genomes are organized into discrete units, the chromosomes, whose uniqueness is defined by their DNA base sequences. The genome includes sequences specifying information for chromosome replication, maintenance, packaging, and transmission; other sequences that with their coding and control regions represent expressed genes; still other sequence familics often regarded as parasitic because of methods of duplication, transposition, and recombination that allow nonorthodox methods of transmission; and many other sequences of unknown function and significance.

Comparative genomics examines properties of genomes from different species. It represents an extension of the classical tradition of the comparative approach in evolutionary biology to questions of genome structure and function. One property of genomes is their complexity spectrum, which describes the distribution of DNA sequences into different copy number classes (single copy, moderately repeated, or highly repeated). A related

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property is the interspersion pattern, which is often summarized by the relative lengths of single- and multiple-copy sequences alternating along an average stretch of the chromosome. Numerous studies in the 1970s demonstrated that different species often have strikingly different complexity spectra and interspersion patterns, which led to questions concerning the significance of the highly repeated sequences.

Some of these sequences are now known to be directly related to the physical maintenance, replication, and transmission of the chromosomes themselves. For example, the chromosomal features called centromeres appear to be essential for proper segregation during mitosis and meiosis. Centromere-specific repeated sequences have been described from a variety of different organisms, and the functional significance of sequence variation has been investigated using yeast artificial chromosome systems (8). The ends, or telomeres, comprise a second important feature of linear eukaryotic chromosomes. Continued maintenance of these ends over the course of many cell cycles poses special challenges because of the unidirectional method of replication by DNA polymerases. Telomere-specific repeated sequences have likewise been described from a few organisms; some of these appear to be quite highly conserved in evolution and are in fact generated not by polymerases but by the remarkable reverse-transcriptase-like mechanism of the enzyme telomerase (8). Other telomere-associated repeated sequences of unknown function are also present, often differing when different genomes or even different chromosomal ends of the same genome are compared. A third aspect of the physical integrity of chromosomes that may be reflected in sequence patterns is the manner in which the exceedingly long DNA strand is coiled and packaged into nucleosomes and higher-order structures, as well as the attachment of these structures to the nuclear membrane. Repeated sequences that seem to be involved in nuclear-membrane attachment have been identified and are called *scaffold sequences* (30). Characterization of these sequences has still not yielded much information regarding interspecific comparisons, although conservation is high enough to allow sequences from one species to bind scaffold proteins from others.

In contrast to repeated-sequence families devoted to the maintenance of the whole chromosome, other semiautonomous elements seemingly function primarily to maintain themselves. Copia, gypsy, mariner, P-elements, and others are known from *Drosophila* spp. Some of these encode transposases and reverse transcriptases, biological activities permitting these sequences to spread from location to location within the genome. These elements are also transmitted by the orthodox mechanisms of mitosis and meiosis, by virtue of being physically integrated into the eukaryotic genome. Although semiautonomous sequence elements may be ubiquitous in eukaryotes, they appear to evolve so rapidly that the elements harbored by distinct species share little

sequence similarity. Moreover, there seem to be great interspecific differences in the amount of such elements in the genome, for reasons not wholly understood. The bizarre evolutionary dynamics of these elements add another level of complexity to comparative genomics.

Somewhat simpler is the comparative genomics of single-copy sequences. By definition, a single-copy sequence has a unique map location in a given haploid genome. With the exception of intraspecific chromosomal inversions and translocations, this uniqueness of map location extends to all the genomes in a given species, and thus a species-specific linkage map can in principle be constructed for all known single-copy sequences. Provided that homologous single-copy sequences can be identified in another species, comparative linkage mapping will reveal to what extent ancestral combinations of genes have been conserved in the evolutionary divergence of the two species. Currently, most comparative linkage mapping in higher eukaryotes involves comparisons among mammals or among drosophilid flies; meaningful comparisons between widely different taxa can seldom be made.

We step back towards increased complexity when considering groups of single-copy genes within a single species that share high sequence similarity. These groups represent a gene family, which is usually classified by a conserved sequence feature (e.g. homeobox genes) or common function of the gene products (e.g. serine proteases, globins). The number of genes in the family and their genomic distribution pattern (clustered or dispersed) must also be considered when comparing linkage relationships.

The Scope of This Review

Unfortunately, for most of the levels of organization described above, no data base is available for comparisons between different insect species. Although the insect *Drosophila melanogaster* is one of the many model systems on which such studies are based, the most informative comparisons at most of these levels usually involve noninsect systems such as yeast, humans, protozoans, and maize. This situation is not likely to change in the near future, because researchers will continue to exploit the powerful genetic tools provided by these model systems.

The one level of genomic complexity that does provide a data base for comparisons between different insect species is the organization of single-copy genes, as represented in their genetic linkage maps. This is the subject of the present review. Currently, linkage maps have been constructed for almost 30 species of insects, and the availability of improved methodologies suggests that this number will double in the next 10 years. This review summarizes the current knowledge of insect genetic linkage maps and provides some suggestions for the coordination of future research in this area.

Such a data base for insects results, first, from many insects having been

the subject of detailed genetic studies because of their economic importance or their tractability as model systems. This has led to the independent construction of classical linkage maps for many species, providing the raw material for the comparative approach. Second, the presence of polytene chromosomes in certain cells of many of the higher Diptera allowed investigators to represent genome structure graphically, by diagrammatic maps of chromosome structure, and to compare these diagrams for several different species. The advantage of this approach is that it bypasses the need for genetics. Even if no genes had been mapped in drosophilids, a reasonably complete picture of their chromosomal evolution could have been constructed, as has been the case with other higher Diptera. But the additional genetic information available in drosophilids has clarified this picture considerably.

The comparative study of genetic maps has always been guided from the perspective of organic evolution. For each species, the existing pattern of genomic organization is the product of the ongoing dynamic process of genomic reorganization, which includes many mechanisms: rearrangements, duplications, and deletions of pieces of DNA ranging in size from chromosomal segments down to individual genes and even shorter repeated elements. Changes in these structural aspects of genomes in turn have led to changes in emergent, functional properties of genome organization and expression, which themselves constrain future avenues of evolutionary change. We hope to eventually infer some general properties of the dynamic process by a comparative study of its many products, and to reconstruct the history of the process in certain specific cases of evolutionary interest. Insects possess a sufficiently diverse collection of genetic systems and patterns of chromosomal organization to make this comparative approach worthwhile. The central thesis of this review is that comparative genomics of the insects is important and feasible.

INSECT SPECIES WITH LINKAGE MAPS

We turn now to the raw material for this comparative approach: linkage maps for individual insect species. For the purpose of this review, a generous criterion for the existence of a linkage map is used: 75% or more of the chromosomes must be marked with one or more markers. Table 1 lists 27 species for which genetic linkage maps have been published, along with some information concerning map completeness and the types of markers employed. The effort has been heavily biased in favor of the Diptera, which account for 21 of the entries. Six of these are species of *Drosophila* (certainly an incomplete list), nine are mosquitoes, three are carnivorous flies, two are fruit flies, and one is the house fly. One orthopteran, two coleopterans, two hymenopterans, and one lepidopteran make up the remainder.

The haploid chromosome number of each species is listed in Table 1, as well as a breakdown into autosomes and sex chromosomes. [For the culiciform mosquitoes, genotype (Mm or mm) at an autosomal locus determines sex, and heteromorphic sex chromosomes are absent.] The approximate total number of mapped loci is given, and the number of distinct linkage groups identified. Many of the entries in the table deserve special comment.

The classical genetics and cytogenetics of the German cockroach, *Blattella germanica*, has been intensively studied by Ross, Cochran, and their associates over the past 30 years. More than half of the approximately 80 known mutants have been mapped to 11 linkage groups, most of which have been correlated to chromosomes by using translocations (66, 67). The majority of mutants are visible, but genes conferring insecticide resistance and translocation breakpoints have also been mapped. Most are still maintained in laboratory stocks at the Virginia Polytechnic Institute and State University. These are an extremely valuable genetic resource, representing the only comparable genetic material from the order Orthoptera or indeed any of the orders of insects with incomplete metamorphosis, and their continued maintenance should be given a high priority.

The silkworm *Bombyx mori* is the most intensively studied nondrosophilid insect. Its classical genetics began shortly after comparable studies on drosophilids and have kept pace with these studies, aided by the economic importance of silk and the frequent opportunities for noticing morphological aberrations in rearing. This has been fortunate for insect genetics, for in one sense the silkworm is a crop that just happens to be an insect. Much of the primary literature in Japanese and Chinese has been summarized in English-language reviews (21, 82, 83). To date, approximately 180 genes have been mapped to 28 linkage groups, all but two of which contain two or more loci. Thus, it is highly likely that all 28 chromosomes are marked, although correlation of actual chromosomes to linkage groups has been impossible because of the extremely difficult cytogenetics. The chromosomes are small, holocentric, indistinguishable dots, as in most Lepidoptera. Tazima et al (83) list more than 300 mutants, some of which represent multiple alleles at the same locus. Hundreds of stocks are maintained at sericultural and genetic research institutes in Japan, China, India, Korea, Thailand, and other countries. Assignment of new mutants to linkage groups is facilitated somewhat by the occurrence of achiasmatic meiosis in females. Most mapped loci correspond to morphological or physiological abnormalities.

Geneticists have exhibited less of a fondness for beetles than that attributed to the Creator by Haldane (23a), for they have only made linkage maps for two species. Sokoloff summarized the linkage information for *Tribolium castaneum* and *T. confusum* up to the late 1970s (74). More recent studies (7, 18, 75) have consolidated two pairs of linkage groups for *T. castaneum*,

Table 1 Insect species with linkage maps

Species	Pairs of autosomes	Sex determination mechanism[a]	n[b]	Number of linkage groups[c]	Linkage groups correlated to chromosomes?	Mapped loci: Number	Mapped loci: Type[d]	References[e]
Orthoptera								
Blattella germanica	11	XX/XO	12	12	Partly	42	mr	66; 67
Lepidoptera								
Bombyx mori	27	ZW/ZZ	28	28	No	182	me	21; 82, 83
Coleoptera								
Tribolium castaneum	9	XX/XY	10	8	Partly	60	m	73, 74; 7, 18, 75
T. confusum	8	XX/XY	9	6	Partly	20	m	73, 74
Diptera								
Drosophila ananassae	3	XX/XY	4	4	Yes	70	m	51
D. hydei	5	XX/XY	6	6	Yes	86	m	37
D. melanogaster	3	XX/XY	4	4	Yes	3700	all	3; 48
D. pseudoobscura	4	XX/XY	5	5	Yes	70	me	2
D. virilis	5	XX/XY	6	6	Yes	190	m	1; 35
D. willistoni	2	XX/XY	3	3	Yes	70	me	23; 22
Ceratitis capitata	5	XX/XY	6	5	No	9	m	70; 71
Bactrocera dorsalis	5	XX/XY	6	5	No	24	m	45; 44
Glossina morsitans	2	XX/XY	3	3	Partly	12	em	33
Lucilia cuprina	5	XX/XY	6	6	Yes	53	mre	27; 89
Cochliomyia hominivorax	5	XX/XY	6	4	Partly	10	me	81
Musca domestica	5	XX/XY	6	6	Yes	60	mre	38; 49, 50
Anopheles albimanus	2	XX/XY	3	[redacted]	Yes	34	mer	57
A. culicifacies (A)	2	XX/XY	3	[redacted]	No	17	mre	68
A. gambiae	2	XX/XY	3	3	Partly	11	me	56a; 39

A. quadrimaculatus (A)	2	XX/XY	3	3	Yes	22	mer	58
A. stephensi	2	XX/XY	3	3	No	18	mre	62
Aedes aegypti	3	auto.	3	3	Yes	77	mer	53; 55
A. togoi	3	auto.	3	3	No	23	me	79
A. triseriatus	3	auto.	3	3	Yes	30	em	54; 52
Culex pipiens	3	auto.	3	3	Yes	19	mer	5, 42; 19
Hymenoptera								
Habrobracon juglandis	10	h/d	10	8	No	42	m	88
Nasonia vitripennis	5	h/d	5	5	No	47	m	69

[a] XX/XY, XX/XO, ZW/ZZ: For species with heteromorphic chromosomes, female karyotype is listed first. Auto.: Sex in *Aedes* and *Culex* spp. is determined by an autosomal locus (Mm, males; mm, females). h/d: Haplodiploid (haploid males and diploid females).

[b] Haploid number of chromosomes, including X (or Z) for species with heteromorphic sex chromosomes.

[c] Number of distinct linkage groups defined by the collection of mapped loci.

[d] m, morphological (visible) mutants; e, enzyme polymorphism; r, resistance to insecticides. More numerous types are listed first.

[e] Secondary references follow semicolon.

reducing the number of linkage groups from 10 to 8; two chromosomes thus remain unmarked. *T. castaneum* is enjoying a resurgence of genetic attention due to its interesting homeotic mutants (6), and a series of translocation stocks is under construction (7).

The genetic map for *Drosophila melanogaster* is by far the most extensive of any insect species. Considerable progress has also been made towards constructing a physical map, defined as an ordered collection of DNA fragments that collectively cover the entire genome. Merriam et al (48) summarize the methodologies employed for this endeavor and display the status of the physical map aligned with both genetic and chromosomal maps for this species. The other drosophilids listed represent a sample of the linkage maps constructed during the heyday of classical genetics in the 1920s through 1940s, supplemented with more recent information on enzyme loci.

Mosquitoes, important vectors of disease, have been the subjects of intensive cytogenetic study for many years. Progress in genetic mapping has been frustrated by taxonomic complexity and difficulties in rearing, among other factors. Fortunately, the haploid chromosome number is only three, enabling chromosomal coverage of markers in spite of the low number of described mutants. Interestingly, insecticide-resistance genes have played a relatively important role in defining mappable loci. Indeed, the phenomenon of insecticide resistance has provided much of the impetus for genetic studies of this important group of Diptera.

Similarly, the economic importance of fruit flies and flesh flies has stimulated much genetic research on those insects, often resulting in enough information to construct linkage maps. A group preeminently successful in this approach is the CSIRO geneticists who have studied Australian sheep blow flies for several years (89). A prime motivation for genetic studies of fruit flies and mosquitoes has been the desire to devise methods of insect control relying on genetic means, such as sterile translocations, sex-linked lethals, and genetic sexing systems to be employed in mass-rearing efforts. Studies on houseflies in particular have provided the most useful information from insects on the genetic and physiological interaction of different pesticide resistance mechanisms.

Although parasitic Hymenoptera are widely employed as control agents for pest insects, genetics plays a comparatively minor role in these approaches at present. Both linkage maps for Hymenoptera listed in Table 1 were developed, not in response to these applications, but rather as part of the efflorescence of the classical period of genetics. Haplodiploid sex determination in Hymenoptera is especially useful in genetic analysis and map construction. Haploid sons receive a single chromosome complement from their diploid mothers, and all loci segregate 1:1 in the sons of heterozygous mothers regardless of their dominance properties in diploid daughters.

The majority of markers employed to construct the linkage maps of Table 1 are mutants that cause visible alterations in the phenotype. These are typically rare in natural populations and need to be isolated and maintained in special stocks before being mapped. Mapping strategies have included interspecific as well as intraspecific crosses. Linkage is usually detected in the classical manner by the relative rarity of recombinant genotypes compared with parental genotypes; the map distance between two linked loci is estimated by the recombination fraction, with suitable corrections for interference.

Theoretically, the number of linkage groups in a well-saturated map should equal the (haploid) number of chromosomes observed by microscopy. This is the case for most species in Table 1. For some species, correspondences between linkage groups and individual chromosomes have been made by combining cytogenetic and genetic techniques, but this approach is not always possible. Most nondipterans lack polytene chromosomes, and for many groups (especially Coleoptera, Lepidoptera), the metaphase chromosomes are all the same size and impossible to distinguish from one another. Comparison of polytene chromosomes of distantly related Diptera is generally not feasible without additional genetic information, because there are not enough similarities to indicate common chromosomal regions.

Construction of these linkage maps has utilized unique properties of genetic systems to speed the process in particular cases. One such genetic system is distinguished by achiasmatic meiosis in one sex only. Crossing-over between chromosomal homologues never occurs in the sex with achiasmatic meiosis. In many Diptera (but not *Culex* or *Aedes* spp.), crossing over occurs only in females; in most Lepidoptera crossing over is restricted to males (65, 77, 82, 87). Thus, by choosing the sex that lacks crossing over as the informative parent in a preliminary round of crosses, any genes on the same chromosome will show zero recombination, and genes on different chromosomes will show free recombination—a clear-cut result that permits rapid assignment of genes to linkage groups. Subsequent crosses with the opposite sex informative for genes known to be on the same chromosome enable recombination rates and map distances to be derived within each linkage group.

Even in species that lack sex-restricted achiasmatic meiosis (such as Coleoptera and Orthoptera), recombination rates between linked loci are typically different when measured in the two sexes. For example, crossover rates between the same pair of genes on linkage group 4 in the German cockroach vary between 3% for males and 17.5% for females (66). Sex differences in rates of crossing over have been found in vertebrates as well. Whatever the mechanistic explanation for this phenomenon, the practical implications are that linkage data from the two sexes cannot be uncritically pooled for the construction of linkage maps.

Because genetic information is available in the form of a linkage map does

not necessarily mean that the information can be readily used. Many of the morphological mutants originally mapped in Table 1 have been lost. For the others, their utility in future mapping efforts depends on strain availability. A few of the species in Table 1 have well-funded stock centers that can guarantee availability of material, but some are extremely vulnerable in that only a few labs maintain stocks. Most geneticists have found that adequate maintenance of stocks is always threatened by inadequate financial and human resources; this factor should be considered in planning future mapping efforts.

INSECT SPECIES WITH SOME LINKAGE INFORMATION

Linkage information of several loci has been established by crosses for several other insect species, but not by enough to account for all the expected linkage groups. Many of these are described in the sections below. These have been chosen for their taxonomic diversity as well as the different biological questions addressed using the tool of linkage mapping.

Hymenoptera

In the honey bee *Apis mellifera,* three linked pairs of genes for visible mutations are known out of $n=16$ possible linkage groups (85). Isozymes have been studied in *Apis* and other bees but have not been incorporated into the linkage map yet (78). Genetic studies on the honey bee have traditionally been motivated by management considerations of this beneficial insect. More recently, studies have focused on the genetic basis of behavioral differences between European and African races, as the descendents of African bees imported into Brazil continue their inexorable march northward.

Lepidoptera

The Mediterranean flour moth *Ephestia kuehniella* was developed as a model system for developmental and biochemical genetics in the 1930s, and several mutants were described and linkages measured (13). Earlier reports of crossing over occurring in both sexes were not substantiated upon replication (84) and were probably artefacts of the methods used to score the phenotypes (E. W. Caspari, personal communication). Sheppard et al (72) summarized extensive genetic studies (including linkage information) on wing-pattern traits in the mimicry complex of *Heliconius* butterflies. Robinson's book (65) reports on genetic studies on many Lepidoptera, including tests of linkage. Most of these studies provide evidence for absence of crossing over in females, which is also supported by abundant cytogenetic evidence (77). However, a recent study of isozyme linkage in the butterfly *Colias eurhytheme* provided evidence for crossing over in both sexes (12a). Genetic studies on isozymes in the tobacco budworm *Heliothis virescens* have revealed five sex-linked loci,

producing a rudimentary map of the X chromosome in that species (D. G. Heckel, unpublished data). Genes controlling morphology, diapause, and isozymes were mapped on the X chromosome of *Papilio glaucus* (36).

Coleoptera

Sokoloff (73) lists two sex-linked mutations and three linked autosomal loci for the tenebrionid beetle *Latheticus oryzae*.

Diptera

Mustermann has determined linkage relationships among isozyme loci in three additional species of *Aedes* (52, 56). Isozyme mapping has been reported for the fruit fly *Rhagoletis pomonella* (25), an especially interesting species believed to represent an example of sympatric speciation resulting from host-plant race formation. Isozymes have been mapped in *Aedes albopictus* (80), *Drosophila subobscura* (63), and *Drosophila tropicalis* (34).

Orthoptera

Chapco et al (15) summarized linkage information of color and isozyme traits in the grasshopper *Melanoplus sanguinipes,* including five loci on one linkage group and one locus on each of three separate linkage groups. A vitellogenin gene in *Locusta migratoria* was found to be sex-linked (10). Other studies are listed in a bibliography on genetics of grasshoppers and locusts (14).

COMPARISONS OF EXISTING INSECT LINKAGE MAPS

Having exhibited the raw materials for a comparative approach to linkage mapping, we now turn to the four most successful examples of their deployment. Deliberately excluded here are cytogenetic studies in which classical linkage maps did not play a significant supporting role—surely the majority of published comparative cytogenetics.

Within the insects, comparisons of chromosomal and linkage maps for various species of *Drosophila* have received the earliest and most enduring attention. Sturtevant & Novitski's pioneering 1941 study (76) revealed the presence of 6 chromosomal elements or segments within which gene association (though not necessarily gene order) was conserved across 14 distinct species of *Drosophila.* These elements correspond to entire chromosome arms or portions of them. The homologous loci Sturtevant & Novitski used were morphological mutants, which were often strikingly similar in several different species. Extensive genetic studies had already enabled researchers to map many of these mutants in several species. Sturtevant & Novitski used these maps to compare pairs of *Drosophila* species that were too distantly related for comparison solely on the basis of chromosomal banding patterns, which

is reliable only for close relatives. Many workers, relying on both genetic and cytogenetic evidence, built upon this approach of establishing homologies by linkage mapping to give the comprehensive picture of drosophilid chromosomal evolution available today (16).

These comparisons were extended beyond *Drosophila* to other higher Diptera by Foster et al (26), who compared their map of *Lucilia cuprina* to those of *Drosophila melanogaster* and *Musca domestica*. This effort was remarkable because it relied solely on genetic markers and not on a cytogenetic approach, and was possible only because many genes had been independently mapped in each species. Foster et al suggest that the autosomes 2, 3, 4, 5, and 6 in *Lucilia cuprina* are homologous to chromosomes 1, 3, 2, 4, and 5, respectively, in *Musca domestica,* and to 2L, X, 3R, 3L, and 2R, respectively, in *Drosophila melanogaster*. These correspondences were made by identifying morphological mutations producing similar phenotypes in two or all three of these species, as well as biochemical mutations with well-characterized effects, homeotic mutants, allozyme variants, and even insecticide-resistance genes. In many cases, the correspondences made were tentative, but little data have appeared to refute the overall conclusions. The very fact that correspondences between blocks of genes as large as entire chromosome arms is even possible here reveals much about the process of genetic reorganization occurring in the higher Diptera: noncentromeric translocations and pericentric inversions occur relatively rarely compared with centromeric translocations and paracentric inversions. This is the same pattern as originally observed by Sturtevant & Novitski within the *Drosophila* species, and thus one can tentatively conclude that the pattern of chromosomal reorganization is qualitatively similar within and between genera of higher Diptera.

Another notable series of interspecific comparisons has been made by Munstermann and colleagues (52, 55, 56) over the past 10 years in mosquitoes of the genus *Aedes*. All *Aedes* species have three pairs of chromosomes, but the difficulty of obtaining good polytene preparations has precluded the straightforward cytogenetic approach. As previously mentioned, linkage maps of various degrees of saturation have been constructed for 6 species using morphological mutants and up to 20 enzyme loci. Enough enzyme loci have been mapped in all six species to reveal both conservation of linkage within chromosomal elements and some shuffling of elements among chromosomal arms. For example, all linkage relationships are conserved among three species in the subgenus *Stegomyia* (*A. aegypti, A. scutellaris,* and *A. albopictus*). The two linkage groups Pgm-Gpd-Had-Idh2-Est6 and Gpi-Hk4-Odh are conserved in the six compared species (covering four subgenera within *Aedes*). Moreover, the latter linkage group is even conserved across mosquito genera as diverse as *Culex* and *Anopheles* (55). The remarkable feature of this body of work is that the opportunity to practice the comparative approach

motivated the mapping in the separate species, and the choice of homologous loci to map was dictated by the desire to employ them in interspecific comparisons.

The only published attempt at comparing linkage maps for nondipteran insect species is Sokoloff's compilation of homologous genes in *Tribolium castaneum, T. confusum,* and additional beetle species (73). Based on consideration of 21 putatively homologous morphological mutations, and additional cytological data, correspondences between some autosomes of the two species of *Tribolium* can be made. Moreover, some statements concerning chromosomal evolution in the genus are also possible. *T. castaneum* has nine pairs of autosomes, an X chromosome about the same size as the autosomes, and a small Y chromosome. This is probably the primitive chromosomal condition for *Tribolium.* In contrast, *T. confusum* has eight pairs of autosomes and larger X and Y chromosomes than its congener. The large X chromosome of *T. confusum* may be a "neo-X" corresponding to a fusion between the small X chromosome and autosomal linkage group II of *T. castaneum* (7, 15). The homologous "neo-Y" appears to have undergone extensive heterochromatization. This hypothesis is supported by the existence of some loci that are autosomally inherited in *T. castaneum,* but sex-linked in *T. confusum.* At least some of these loci map to linkage group II in *T. castaneum.*

These interspecific comparisons of linkage maps, interesting as they are, nevertheless reveal the severe limitations presented by the paucity of genetic data in insects. Comparisons are still only possible among closely related groups. Meaningful comparisons across insect orders have not been at all possible. The limitations are of two basic kinds: few species mapped and, more importantly, few homologous loci mapped per species that would allow meaningful comparisons across higher taxonomic categories. Fortunately, rapid technological developments have recently supplied insect geneticists with the tools necessary to remedy both types of limitations, as discussed next.

NEW AND IMPROVED TECHNOLOGIES FOR GENOMIC MAPPING

Roughly speaking, the new technologies discussed below fall into two categories—genetic approaches and physical approaches. Although both involve more powerful manipulations of DNA and both produce maps, they do so through different intermediate steps. The genetic approaches are extensions of classical genetics, harnessing new technologies to generate molecular markers that are subjected to linkage analysis. The physical approaches are extensions of classical cytogenetics, in that new methods are used to physically subdivide chromosomes into smaller and smaller regions,

in such a way that knowledge of the hierarchical relationships between the parts and the whole is recoverable.

Classical Linkage Mapping

The main benefits of recombinant DNA technology for classical linkage mapping have been techniques for generating and scoring large numbers of variable marker loci. These enable a multilocus approach to linkage mapping, in which the ratio of genetic information to individuals studied can be maximized. These loci are scored in progeny resulting from crosses in which morphological markers or other molecular markers are segregating, and the data are analyzed using classical linkage analysis, often supplemented by computer programs designed to meet the increased computational demands of the multilocus approach. Foremost among these techniques is restriction fragment length polymorphism (RFLP) analysis and its variants, which have revolutionized human genetics in the past 10 years and are currently being applied to the study of many species of plants and animals, including insects.

The basic steps involved in RFLP analysis include the isolation of DNA from individuals, digestion with restriction enzymes, separation of the fragments on the basis of size by gel electrophoresis, transfer of the DNA to a membrane by Southern blotting, and probing the membrane with a labeled fragment of DNA, which hybridizes to specific target sequences. Individual-to-individual variation in the patterns seen is caused by underlying genetic variation in the target DNA sequence (basepair substitution creating or abolishing restriction sites) or sequence arrangements (insertions/deletions of DNA in the interval encompassed by neighboring restriction sites).

Depending on the type of probe employed, different classes of marker loci are revealed. Most widely employed for mapping are single-copy loci with unique genomic locations; these are visualized when single-copy sequences (either anonymous or corresponding to known, cloned genes) are used as the probe. In many species, probes have been discovered that light up complex, hypervariable patterns consisting of dozens of bands. One class of these are the minisatellite probes, consisting of core sequences of 20–50 nucleotides, found dispersed at numerous sites in the genome as tandem arrays of variable numbers of repeats of the core sequence (41). Another class consists of microsatellite probes, in which the core sequences are two to five nucleotides. These probes reveal an enormous wealth of genetic polymorphism, much of which can be mapped if suitable approaches are employed to distinguish separate loci from one another.

Another technology being employed to generate genetic markers utilizes the polymerase chain reaction (PCR). When sequence information is known for a particular gene, primers can be designed to allow screening and detection of single base-pair changes in a specific region of the gene. A novel variant

of PCR called RAPD [randomly amplified polymorphic DNA (90)] or DAF [DNA amplification fingerprinting (12)] requires no prior sequence information, relying instead on short, single primers of arbitrarily chosen sequence. This technique shows great promise although its technological limitations are currently unexplored.

These methods and others have allowed extremely rapid advances to be made in construction of linkage maps for many species, including mammals such as humans and mice; crop plants such as maize, soybean, tomato, and lettuce; and fungi. Comparable efforts on many insect species are also underway.

Physical Approaches to Mapping

The most conceptually straightforward extension to the classical cytogenetic approach involves the technique of in situ hybridization of cloned gene segments to chromosomes, to correlate physical location with specific DNA sequences. Although hybridization was effectively limited to species with polytene chromosomes for many years, recent development of fluorescent probes is allowing this type of comparative cytogenetics to be pursued in other species, notably mammals. These techniques allow one to determine the (previously unknown) location of a known sequence.

The converse of the above approach is to reach into the nucleus and collect all the (previously unknown) sequence information from a known location. Microdissection of polytene chromosomes has been one method of determining these sequences and has been successfully employed to produce libraries enriched for the contents of particular chromosomal bands in *Drosophila*. In a quantum leap for mosquito genetics, an adaptation of this approach was recently used to construct a low resolution physical map of *Anopheles gambiae* (91). By microdissecting out 54 chromosomal regions, digesting the DNA with a restriction enzyme, ligating the fragments to linkers, and amplifying the fragments by PCR, these investigators generated a set of 46 pooled probes, accounting for about 80% of the total genome. Creating an array of these probes immobilized on filters enables a more convenient approach to in situ hybridization than using the actual chromosomes as hybridization targets.

The most intensively pursued physical approaches to genome analysis in *Drosophila melanogaster* are the bottom-up methods, which start with yeast artificial chromosome (YAC) or cosmid libraries and attempt to conceptually link them together to account for the entire genome. Merriam et al (48) recently reviewed the status of these projects. Comparable effort will probably never be spent on any other insect species, with the possible exception of the silkworm moth. Yet the information emerging from these projects will be of enormous value in all future comparative approaches to linkage mapping in insects.

Another type of physical technique that may be more widely applied to insects uses PFGE (pulsed field gel electrophoresis) to physically separate chromosomes or chromosome fragments. This technique at least has the potential to be applied to species lacking polytene chromosomes, as illustrated by a recent study in silkworm moth in which a chromosomal fragment was physically separated from the main chromosomal complement (29).

Development of these physical techniques is a rapidly growing area of genomics, as described in several recent reviews. Ashburner (4) discusses these specifically in regard to linkage mapping in insects. The few examples discussed here illustrate that these methods can be applied to insects and are likely to have a significant impact.

Construction and Extension of Linkage Maps

Overall, the appreciation of the power of the genetic approach, and the utility of linkage maps in particular, is increasing among entomologists. Inspired (or repelled) by the Human Genome Project, many groups are actively engaged in constructing RFLP maps in a variety of different insect species. Several laboratories have recently begun employing RFLPs, in situ hybridization, pulsed field gel electrophoresis, and other molecular techniques in augmenting the linkage map for *Bombyx mori* (32; M. Goldsmith, personal communication). This work should proceed rapidly because of the importance of sericulture to many countries and also for its use in studying many basic biological processes for which the silkworm is an ideal model system. Combined genetical and cytogenetic thrusts in mapping the genome of *Rhagoletis pomonella* have begun, partly to unravel the genetic basis of host specialization that may be so important in speciation (24; G. Bush, personal communication). RFLP mapping of the honey bee genome is currently underway (20). Inheritance of RAPD markers has also been investigated in honeybee, whose haplodiploid genetic system enabled the detection of a novel type of RAPD polymorphism (40) and will greatly facilitate mapping efforts. Isozyme loci are being mapped in the Hessian fly (W. C. Black IV, personal communication) to aid in the mapping of virulence genes that enable this pest to damage wheat. Isozyme (D. G. Heckel, unpublished data) and RFLP markers (92, 93) have been identified for some, and RAPD markers (L. C. Gahan & D. G. Heckel unpublished) have been identified for all, the linkage groups of the tobacco budworm *Heliothis virescens* to aid in mapping of insecticide-resistance genes in this highly adaptable species.

Clearly, the rich variety of new techniques will greatly facilitate the construction of linkage maps for insect species. However, the new profusion of maps may or may not be suited to the needs of comparative linkage analysis. For intraspecific linkage mapping, any Mendelian locus can contribute positional information, irrespective of the type of trait encoded by the locus.

But for interspecific linkage comparisons, the loci being compared must be homologous. This requirement has important implications for the advancement of comparative genomics and deserves special attention.

HOMOLOGOUS MARKERS FOR WIDE-SCALE COMPARISONS

The Importance of Homologous Loci

Consider two species that are evolutionary descendants from a common ancestor. Two loci (one in each of the descendant species) are considered to be homologous if they are both derived from the same locus in the ancestral species. This property of homology is fundamentally important to all inferences we are able to make utilizing the comparative approach in studying evolution. Comparative genomics is no exception to this general principle, and so the accurate determination of homology is crucial to successfully understanding the evolution of genomes. Apart from this fundamental conceptual importance, the need for homology has practical consequences as well, since it affects the rate of progress of our understanding.

Genetic variation, in the form of marker loci with scorable phenotypes, provides the raw materials for the construction of a linkage map for a given species. In the days of classical genetics (for *Drosophila, Habrobracon,* and *Nasonia,* and even now for many mosquitoes, *Blattella,* and *Tribolium*), one of the rate-limiting steps in linkage map construction was the isolation of suitable markers. The necessary raw materials for the comparison of linkage maps are even rarer—they are marker loci that can be identified as homologous in two or more species. If the process of identifying markers within a species is random, then the rate of production of homologous markers in several species is extremely low. The speed of discovery and mapping of homologous loci is the rate-limiting step in the comparison of insect-genome organization. Furthermore, because homology is usually easier to demonstrate among close evolutionary relatives than among distant ones, the difficulty of finding homologous loci limits broader comparisons more than it does taxonomically narrow ones.

Although homology is a crucial aspect of comparative genomics, it is usually ignored at most other levels of genetic study. Therefore, we discuss some different types of markers from this perspective.

Classes of Homologous Markers

MORPHOLOGICAL MUTANTS Mutations causing visible changes in the phenotype have been used to compare linkage maps within *Drosophila* and *Tribolium*. For example, eye-color mutants in various species of Diptera have

been suggested to be homologous, due to similarities in the underlying biosynthetic pathways for eye pigments. Morphological mutants are generally comparable only between close relatives that have great phenotypic resemblance to begin with. After all, one *Tribolium* looks pretty much like another, as the species name *confusum* testifies. Generation of these variants relies on mutagenesis or on picking rare, naturally occurring forms out of large populations. Because most aspects of the morphological phenotype are several steps removed from the underlying genes, validity depends heavily on homology of the entire developmental pathway producing the phenotype in different species. Moreover, different genetic changes may produce similar alterations in phenotype, leading to pseudohomology. These difficulties have been recognized by most investigators who have used morphological mutants as homologous markers. Inferences based on these types of loci are most reliable when several phenotypically independent markers are linked in one species, and these linkages are conserved in another species. In such cases, although the validity of each single marker might be called into question, one assumes that enough true homologies exist to support the truth of the comparative linkage hypothesis being tested.

For comparisons across insect orders, more than one or two morphological mutants will probably not be useful. Thus, the two insects with the best linkage maps—*Drosophila melanogaster* and *Bombyx mori*—are currently useless for comparative genomics at the inter-order level, because the vast number of morphological mutants mapped in the latter are not homologizable to any in the former.

INSECTICIDE-RESISTANCE GENES Because of the demands of modern agriculture and disease control, insecticides are a common selective pressure in the environment of many insect species. Natural selection, in the form of differential ability to survive high doses of these toxins, thus prescreens populations for a particular class of mutants that can be useful for comparative linkage mapping in insects. These mutants will be homologous if the underlying physiological resistance mechanisms are similar; but this homology must be experimentally established first because of the many ways an insect can become resistant to an insecticide—the insect can detoxify it, not allow it to penetrate the cuticle, excrete it, sequester it, behaviorally avoid it, or alter its target site. The most likely class of homologizable insecticide-resistance mechanisms are mutations in genes encoding the target sites, such as acetylcholinesterase (for organophosphates and carbamates), sodium channels (for DDT and pyrethroids), GABA receptors (for dieldrin), and midgut membrane proteins (for *Bacillus thuringiensis* endotoxins). The ever-increasing incidence of resistance (31) promises to furnish a steady stream of this

type of homologous marker for comparative genomics while considerable progress is made in the study of the resistance mechanisms. Currently, insecticide-resistance genes have been mapped in *Blattella, Drosophila, Lucilia, Musca,* various mosquitoes, *Tribolium,* and *Heliothis*. This list includes all the insect orders in Table 1 except Hymenoptera. A gene conferring dieldrin resistance in *Drosophila melanogaster* was recently cloned; this gene may possibly be homologous to dieldrin-resistance genes in sheep blow fly and house fly (26).

ISOZYMES In the mid 1960s, population geneticists discovered that subjecting a crude homogenate of insect cells to gel electrophoresis, and staining the gels for specific enzymes using modifications of histochemical techniques, provided a breathaking vista onto undreamed-of levels of genetic variability in natural populations. This new view was possible because allozymes—allelic forms of the same isozyme—were easily distinguishable by their mobility differences. Although these techniques were largely eclipsed by even more stunning advances in recombinant DNA technology, one should remember that the reasons summarized by Lewontin (43) for the utility of isozyme electrophoresis in studying genetic variation within populations are the same reasons for its utility in comparative genomic analysis. Thus, naturally occurring allozymic variation in several loci representing a more or less random sample of the genome, which is weakly to highly polymorphic in most populations, provides the polymorphism necessary for linkage mapping in any species, whether a classical map exists or not. Presence of this variation in natural populations precludes the necessity of maintaining carefully guarded stocks, because if lost, the variation can always be resampled from the field. Because most of the biochemical pathways in which these enzymes participate are conserved in most prokaryotes and eukaryotes, isozymes are also ideal candidates for homologous genes. Note that the requirements of comparative genomics are different from the conventional use of isozymes as characters in insect systematics, where enzyme mobility (not gene location) is the characteristic being compared and monomorphism within species is the most desirable quality.

The potential for the application of this type of homologous marker to comparative genomics of the insects is enormous. Allozyme polymorphisms have been detected in agricultural, medical, ecological, and evolutionary studies in an extremely large number of species, with numerous representatives in every major insect order (47). As described above, they provided many of the markers mapped in Table 1 and furnished the basis for broad comparisons across mosquito genera and among various higher Diptera. In light of this

potential, it is surprising that so few enzyme loci have been mapped in silkworm moths, *Tribolium,* cockroaches, and Hymenoptera.

DNA SEQUENCES Because of the enormous information content of even short DNA molecules, the physical principle used in detecting variation at a particular locus is the same used to establish homology of loci in different species, namely that formation of a duplex by DNA-DNA hybridization is stable only when sufficient sequence similarity is present. Taking the operational definition of homology as sequence similarity of a certain percentage level, in principle the homologue corresponding to a particular cloned single-copy gene in one species can always be found in another species, provided the second species has one and only one homologous sequence. However, the effort involved may be prohibitive if this must be done separately for each DNA-based marker employed in a linkage map.

In practice, the methods currently used to generate DNA-based markers for linkage maps may not lend themselves readily to a comparative approach. This applies particularly to the so-called anonymous single-copy RFLPs, defined by randomly chosen sequences from a genomic library, whose identity and function are unknown. This type of RFLP accounts for most of the markers on RFLP maps of well-mapped species such as human, maize, tomato, etc. Although extremely useful in genetic studies of individual species, these maps cannot be directly compared with one another, because homologies between different sets of anonymous markers have not been established.

The alternative approach is to map RFLPs defined by cloned genes of known function. More than a hundred sequences of known genes have been obtained from *Drosophila* (3) and could be used to define RFLPs in other insect species. Homology is still an issue, however, because there is no guarantee that all of these will show enough sequence similarity to hybridization targets in different insect orders to be useful directly in Southern blots. The intermediate step of isolating the homologous gene from a library of the different order may be necessary. This strategy will probably be employed in the more intensively studied species such as silkworms and *Tribolium.* For interspecific comparisons within families or orders, it may be possible to use heterologous probes for Southern blots. A successful example of this in plants is the use of probes derived from tomato to construct a map for potato (9).

DNA-based methods of generating polymorphic markers that light up multiple loci will not be useful in comparing linkage maps of different species. This includes microsatellite and minisatellite DNA fingerprints and RAPD markers. Although these are potentially the most useful in the short term for constructing a linkage map, they are the least useful in the long term from the perspective of this review.

DNA-based homologous markers will be most useful in groups amenable to the physical approaches discussed above. Once different regions of the genome are immobilized on filters, the process of screening them can easily be automated, as the proponents of laboratory robotics remind us. Perhaps every insect comparative genomicist will require a personal robot to remain competitive in the field by the next time a review on this subject is written.

Universally Homologous Single-Copy Marker Systems: A Candidate

The classes of marker systems discussed above each have their advantages and disadvantages for single-species linkage mapping. Is there a single class that is most useful for comparative insect genomics? I would say yes—gene-enzyme systems. These systems are easily studied at the level of the protein product by widely accessible techniques readily applied to members of any insect group. They are mostly encoded by single-copy genes that have been conserved during evolution because of the essential functions their products play in the metabolism of the cell. Progress made in cloning many of the genes, if not in *Drosophila* then at least in vertebrates, makes this class of markers ultimately accessible at the DNA level as well.

Utility of this class of markers for comparative genomics does not require that all insect maps consist solely of them, but only that they should always be a part of insect genetic maps. Efficiency in using them in mapping requires not that DNA-based methods be avoided, but that mapping first be attempted using the enzymatic detection methods, which are faster, cheaper, and safer.

Still, the issue of true versus false homology must be rigorously addressed for this system of markers as well, for at least four reasons. (*a*) Not all proteins with the same enzymatic function are evolutionarily homologous. The protein sequence of alcohol dehydrogenase (ADH) of *Drosophila* species is markedly dissimilar to ADH sequences from plants, vertebrates, and fungi, all of which are more similar to one another than any are to *Drosophila* ADH. The enzymatic function has apparently been taken over by another protein in *Drosophila,* and the extent to which other insect species share this property with *Drosophila* is currently unknown. (*b*) Recent gene duplication can produce too many homologues in some species, leading to problems in interpretation. Amylases are known to have undergone gene duplications in insects and other organisms. If the duplicate locus is tightly linked to the parent locus, as is often the case, relative linkage information may be unaffected, but if the duplicate locus is the result of retrotransposition, more analysis will be needed. (*c*) Many isozymes exist in forms encoded by distinct nuclear loci, the products of which are differentially translocated to cytoplasm or mitochondria. Cell fractionation experiments may need to be carried out

to positively identify the mitochondrial form of one species with the true homologue in another species. Fortunately, this is not as much a problem for insects as it is for plants, in which cytoplasmic, mitochondrial, and chloroplastic forms may exist for many enzymes. (*d*) Some enzymes are easier to homologize than others. Triosephosphate isomerase, phosphoglucomutase, and glucose-6-phosphate dehydrogenase each play well-defined roles in glycolysis and, when assayed using their normal physiological substrates, exhibit unique and simple staining patterns. Esterases, phosphatases, and peptidases on the other hand are usually stained for using artificial substrates, and these typically reveal multiple forms that may be tissue specific or restricted to a particular developmental stage. Additional criteria will need to be used to firmly establish homologies among the last class named. Ultimately this may require a DNA-based approach sensitive enough to distinguish different members of the same gene family.

So long as homologies are verified, then gene-enzyme systems responsible for the many steps of intermediary metabolism should be the most useful single class of markers in the expansion of insect genomics over the next 10 years.

TOWARDS A COMPARATIVE GENOMICS FOR INSECTS

Maximizing Map Comparability

With this information in mind, I recommend four ways of making existing and future linkage maps more comparable to one another in order to serve the needs of comparative insect genomics.

1. In all species with a reasonably complete map based on morphological markers, enzyme loci should be incorporated into the map. Existence of some map information will make this easier than starting from scratch. Many species already offer abundant evidence of enzyme polymorphism, and yet the loci remain unmapped. Individually, enzyme loci are relatively cheap, easy markers to score. Collectively, some organization and experience are needed to routinely score large samples at many enzyme loci. For insect geneticists with available stocks and the ability to do crosses, but who lack the technical expertise for enzyme electrophoresis, there are established labs specializing in electrophoretic analysis that often offer services on a contract basis. Collaborations should be encouraged. This work is especially needed for the single representatives of different insect orders in Table 1: *Blattella, Tribolium, Nasonia,* and *Bombyx*. Furthermore, there are still many enzyme polymorphisms that have not been mapped yet in *Drosophila melanogaster*. It is unlikely that the allozymic variation necessary for this mapping will be found in inbred lab strains. Field collections will provide one source. Forced

interspecific hybridization may provide another—it was especially useful in the anopheline studies (55) and in mapping in *Heliothis* spp. (92).

2. In species in which population studies have shown abundant enzyme polymorphism and in which controlled matings and enough lab rearing is possible for three-generation families, inheritance and linkage studies should be carried out. Much can be learned by analyzing just mothers and offspring, if a single mating can be assumed or sufficiently many alleles are present (86). The Lepidoptera in particular seem to have a lot of potential for this type of analysis. Also, in honey bees allozymes were recently used to study gene introgression of African and European populations in South America. These polymorphisms could easily be mapped. Linkage information from species with abundant enzyme polymorphisms may still be useful in comparative genomics, even if complete maps are never constructed for all of these species.

3. In insect species with RFLP maps currently under construction, at least some mapped *Drosophila* genes should be used as probes. The RFLP map need not be limited to these genes, but the existence of at least some homologues will help in future interspecific comparisons. For example, Regier et al (64) recently screened numerous cloned *Drosophila* genes against Southern blots of various lepidopteran species and found some that revealed identifiable, single-copy homologues. This approach should maximize the number of homologues mappable by either classical or physical methods.

4. Existing and new linkage maps should be submitted for publication to *Genetic Maps* (60), published every few years by Cold Spring Harbor Press and edited by Stephen J. O'Brien. The latest (fifth, 1990) edition contains hundreds of genetic maps, from viruses to humans, but includes only seven of the insect species in Table 1.

Linkage Studies in Mammals as a Role Model

In charting the future course of insect genomics, progress in mammalian comparative genomics can serve as a guide. Although mammals exhibit a less interesting variety of genetic systems than insects, the distribution of mapping effort has been spread around a little more evenly, and as a result, far more interesting comparative material is presently available. Techniques employed include cytogenetics, somatic cell genetics, and RFLPs, which have enabled qualitative and quantitative comparisons of linkage groups in different mammalian families (61). Another interesting point of comparison is that humans are to mammals as *Drosophila melanogaster* is to insects. It is instructive to see how genetic information extracted from detailed studies of a single species can be applied to broader comparative studies of several species.

Utility of Comparative Genomics in Evolutionary Studies

So far this review has addressed the demands of the infant comparative genomics. A worthwhile conclusion is a consideration of what this discipline may contribute to other areas of insect evolutionary biology in the next 10 years. One beneficiary will be studies of insecticide resistance. We still do not understand why some species evolve resistance rapidly to all classes of insecticides with which they are challenged, while others remain susceptible. Comparative ecological, physiological, toxicological, and biochemical studies have shed some light on this question, but comparative genomics promises to shed much more. With linkage maps developed for major pest species, investigators may be able to track allele-frequency changes in insecticide-resistance loci occurring in the field with far more precision than is currently available, an advance that will offer an unprecedented opportunity to observe the evolutionary response of different species to the same selective pressure at the same time and that hopefully will contribute to the more rational and judicious use of insecticides.

Another area in which comparative genetic data on many insect species is needed is the systematic study of the genetic basis of reproductive isolating mechanisms. As Coyne recently pointed out in a lucid review (17), an abundance of theories concern different genetic modes of speciation, but little empirical data are available to evaluate them. Forced interspecific crosses are one method by which the genetic basis of existing species differences can be dissected, so long as the chromosomal contributions of the two species can be distinguished using markers. A promising start on this research program has been made in the genus *Drosophila;* whether similar results will be obtained in different insect orders remains to be seen.

Finally, comparative linkage mapping can serve as a foundation for extending other levels of genome analysis and comparison into the Insecta. Perhaps we can begin to understand how the vast array of genetic systems in insects evolved from a common ancestor. Consider the Diptera and the Lepidoptera, for example. Most Diptera have a few large eucentric chromosomes and heteromorphic sex chromosomes with heterogametic males, and crossing over is restricted to females. Most Lepidoptera have many small holocentric chromosomes and heteromorphic sex chromosomes with heterogametic females, and crossing over is restricted to males. What was the ancestral sex-determination system? Do the small chromosomes of Lepidoptera have to be holocentric? Why is crossing-over among homologous autosomes restricted to the homogametic sex? Does having 10 times as many telomeres per cell as dipterans affect the sequence-complexity spectrum of lepidopterans? Are there specific functional centromeric sequences dispersed

over the chromosomes of Lepidoptera, but localized in the centromeres of Diptera? What is the interaction between the evolutionary dynamics of transposable elements and the architecture of the host genome? These fascinating and persistent questions represent the tip of the iceberg of the diversity of genetic systems in insects (e.g. meiotic systems in coccids) (59). The opportunity to study the evolution of genetics, itself, provides the most powerful motivation for the young and growing science of insect comparative genomics.

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