

## **WORKING GROUP PRIMATE GENETICS**

**Head of Working Group:** Dr. Hans Zischler

### **General research objectives**

Research carried out by the Primate Genetics Group can be subdivided into three thematic areas. The first is concerned with primate-relevant phylogenetic issues that are worked on with molecular methods. In the second research area mitochondrial and tandem repetitive nuclear DNA segments are characterised for different primate infraorders to be used as highly polymorphic marker systems. These markers are employed in the analysis of social systems and the genetic heterogeneity of a species, with the latter representing an important indicator for its ability to survive over evolutionary time scales. The third thematic area falls into the field of immunogenetics and focuses on the genetic analysis of the histocompatibility complex mainly in rhesus monkeys.

### **Phylogeny of primates**

The knowledge of an undisputable phylogeny of the primates and their phylogenetic affiliations to other mammalian orders forms the basis for differentiating between homologies and convergences in the morphological, physiological and behavioural character evolution. With the availability of the complete human and mouse genome sequences it will become increasingly important to trace back the evolution of DNA stretches (and of course also their function) in taxa that are more closely related to humans. The character states in different primates represent different realisations of the evolutionary process along the lineage leading to humans and their comparison allows analysis of character alterations that take place at a higher evolutionary rate and with more precision than is possible in human-mouse comparisons. Despite the importance of these issues for comparative biological and biomedical primatology, numerous phylogenetic questions in the mammalian order of primates remain unsolved to date. These particularly concern recent branching as well as deep splitting events and are thus concerned with the origin of primates as a whole.

The Primate Genetics Group aims to contribute to the solution of these problems through an approach in which phenetic and cladistic analyses are carried out. The latter approach is based on providing evidence for the common presence of non-homologous recombinations, which involve the transfer of nucleic acids from the mitochondria to the nucleus as well as transpositions of nuclear SINEs (short interspersed nuclear elements). By this approach a cladistic framework will be initially established, which will then be complemented by classic sequence analyses particularly of the deep splits. Beyond their use as markers, several aspects of comparative nuclear / mitochondrial sequence evolution are also being considered.

### **Population genetics**

Our main focus in this area is on determining possible causes of the micro-evolutionary forces of drift and migration. We are particularly interested in the

question of how spatial separation of subgroups, their effective size and gene flow among one other is influenced by the respective social system of a species as well as by the ecological parameters of the habitat. Using molecular, neutral markers, relationships between individual group members are being investigated as well as the genetic structures and (historic) demographic events within primate populations. In the transitional stage between microevolutionary analyses and interspecific phylogenies, sequence distances between geographical distantly located primate populations are analysed and interpretations on the subspecies/species level of the respective populations are carried out on the basis of a phylogenetic species principle. The Primate Genetics Group uses two rapidly evolving genetic marker systems: mitochondrial DNA (mtDNA) and microsatellites consisting of short, reiterated sequence motifs, mainly dinucleotides. Mitochondrial DNA markers, which are maternally inherited, allow a separate analysis of the female component of intraspecific phylogeny and population genetics, while nuclear, biparentally transmitted microsatellites reflect the biological history of both sexes.

Comparing the two marker systems can yield insights into a possible asymmetric migration behaviour and the population history of the sexes. Since such comparisons only permit an analysis of those populations with contrasting gender-specific population histories, Y-chromosomal markers of the non-recombining chromosomal regions will increasingly be isolated and incorporated into genetic population analyses in future.

### **Immunogenetics**

The area of immunogenetics is primarily concerned with the clarification of the genetic structure of the Major Histocompatibility Complex (MHC) of rhesus monkeys, with the development of typing methods for the highly polymorphic MHC-genes as well as with the identification of MHC-genes, that influence the disease progression of AIDS in rhesus monkeys. Moreover, MHC genes of other non-human primates, e.g. lion-tailed macaques (*Macaca silenus*) and long-tailed macaques (*Macaca fascicularis*) will be analysed increasingly.

### **Structure of department**

The Working Group consists of 3 scientists, 5 postgraduate students, 2 undergraduate students and 4 technicians, with one scientist, four postgraduates and two technicians funded through external projects.

#### Scientists

Dr. Uta-Regina Böhle  
Dr. Ulrike Saueremann  
Dr. Jürgen Schmitz

#### Technicians

Mareike Hausmann (01.07.00-)  
Martina Ohme  
Claudia Schwiegek  
Nathalie Vigon

#### PhD students

Anja Blankenburg (01.10.00-)  
Andreas Hapke  
Thorsten Mühl  
Christian Roos  
Silke Singer

#### Undergraduate students

Alexander Fischer (-01.04.00)  
Oliver Piskurek

## **Progress during the year**

### **Phylogenetics**

With respect to the cladistic approach, our aim is the clarification of phylogenetic relationships within and between primate infraorders using non-homologous recombinations. This involves the characterisation of interorganellar transpositions from mitochondria to the nucleus as well as transpositions of repetitive elements within the nucleus. The overall aim is a comprehensive analysis of presence and absence of transposed DNA sections across different species. Apart from using these markers in cladistics, we are also exploiting mitochondrial sequences that transposed to the nucleus in the comparative analysis of nuclear and mitochondrial sequence evolution. Here, nuclear insertions of mitochondrial DNA provide an opportunity for carrying out these comparative analyses on homologous DNA sequences.

#### *Sequence evolution*

During the report year, the approach of comparative analysis of the sequence evolution in the nucleus and mitochondria which was first done for the non-coding mitochondrial control region (see annual report '99) was extended to the rRNA-specifying genes of primates. The aim of the study was to characterise the position-specific substitution rate in context with the secondary structure and thus to study the influence of negative selection on the sequence evolution of a functionally constrained gene product. Moreover we wish to follow the course of consecutive compensatory substitutions during primate evolution, which are assumed to maintain or restore the respective secondary structures and can therefore be regarded as being positively selected. For this, we have defined nuclear integrations of mtDNA in humans by querying the database, and characterising the cross-species distribution (presence/absence pattern) of the respective integration in different primate taxa and sequencing the respective orthologues. By interpreting these data in context with the mitochondrial homologues we hope to establish more accurate models of sequence evolution of the primate mitochondrial rDNAs.

Moreover we started to characterise the methylation pattern of a nuclear insertion of mtDNA. In comparison with the unmethylated, homologous mitochondrial sequences, we would like to deduce, to what extent a nucleus-specific mutational mechanism, e.g. the instability of methylated CpG-dinucleotides influences the fossil character of nuclear insertions of mitochondrial DNA.

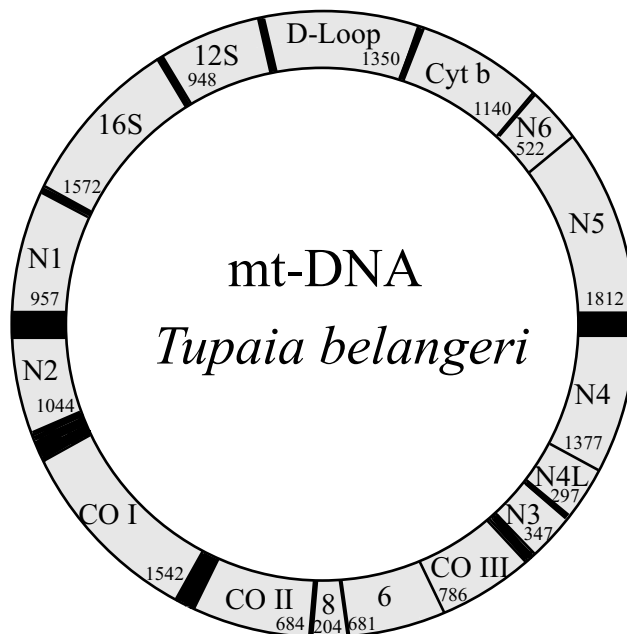
During the report year, the Primate Genetics Group analysed both the infraordinal and the interordinal phylogenetic affiliations of primates by means of classical sequence analyses as well as molecular-cladistic approaches.

#### *Primates and their closest relatives*

One of the most basic questions in primate phylogeny as related to the taxonomical hierarchy of the analysed taxa is to clarify the phylogenetic affiliations of primates to other eutherian orders, and thus to delineate the origin of primates. Gregory (1910) classified the primates together with the eutherian orders Dermoptera, Chiroptera and the members of the genera *Tupaia* and *Ptilocercus*, which are currently included in the order of Scandentia into the superorder "Archonta". This pro-

posal was and still is controversially discussed, however it represents the classical hypothesis on the possible sister-group-relationships of primates to other mammals, which can be tested e.g. at the molecular level. Concerning the Archonta-hypothesis our contribution in the report year has to be seen in context with the examinations carried out by Nikaido et al. (2000), Pumo et al. (1998) and Teeling et al. (2000). In these analyses the phylogenetic positions of micro- and megachiropterans (bats and flying foxes) were determined based on nuclear as well as complete mtDNA sequences. The results of these analyses concordantly show for both the mitochondrial and nuclear data sets, that the Chiroptera constitute a monophyletic group, however they are not closely related to primates. In contrast, the complete mitochondrial genome data suggest that the Chiroptera are phylogenetically more closely related to the ferungulates (Carnivora, Perissodactyla and Cetartiodactyla) as compared to primates.

Thus the question about the phylogenetic affiliation of Scandentia, representing another Archonta-member, to primates emerges. The uncertainty about the phylogenetic classification of Tupaia is reflected in the various systematic classifications attributed to this taxon over the years and can be explained by the complex mixture of plesiomorphic and apomorphic morphological characters detectable in representatives of the Scandentia, the lack of adequate fossil material, mainly pertaining to the Tupaiidae and the long independent evolutionary history of the Scandentia. To answer the question for a possible phylogenetic affiliation of Scandentia to primates on the molecular level, we sequenced the complete mitochondrial genome of a Scandentian representative, *Tupaia belangeri*.



*Schematic representation of the mitochondrial genome of Tupaia belangeri. The genomic organisation corresponds to the typical mammalian mitochondrial genome and contains information specifying the mitochondrial, ribosomal RNA-components (12S and 16S), several polypeptide subunits of the respiratory chain (N, NADH dehydrogenase; CO, Cytochrome oxidase; Cytb, Cytochrome b; ATPase 8 and 6), tRNAs (depicted by bars) and the control region (the term displacement loop is used synonymously) which contains the promoters of the both strands and the replication origin of the H-strand. The length of the coding regions is given in bp.*

The mitochondrial genome of *T. belangeri* is 16,754 bp in length and shows no obvious deviation from the general organisation of eutherian mitochondrial genomes. Characteristics such as, for example, usage of different start codons, incomplete stop codons, overlapping coding regions, or the presence of tandem repetitive DNA in the mitochondrial control region are within the expected variability. To examine a possible close evolutionary relationship between the orders of Scandentia and primates, complete mitochondrial genomes of archontan representatives (primates and bats), ferungulates, guinea pig, armadillo, two representatives of the rodents and the hedgehog were compared to the complete mtDNA of *Tupaia belangeri*. The opossum sequence was used as an outgroup. Phylogenetic reconstructions on the basis of 12 H-strand encoded, concatenated genes yielded further molecular evidence against an Archontan monophyly. Phylogenetic reconstructions applying Maximum Parsimony, Maximum Likelihood and distance based algorithms could not uncover a sistergroup relationship of primates and Tupaia as a representative of the Scandentia. Combining our mtDNA based results with interpretations as obtained from nuclear sequence analyses published by other groups we can hypothesise, that the order of Scandentia exhibits a closer phylogenetic relationship to the Lagomorpha as compared to the primates.

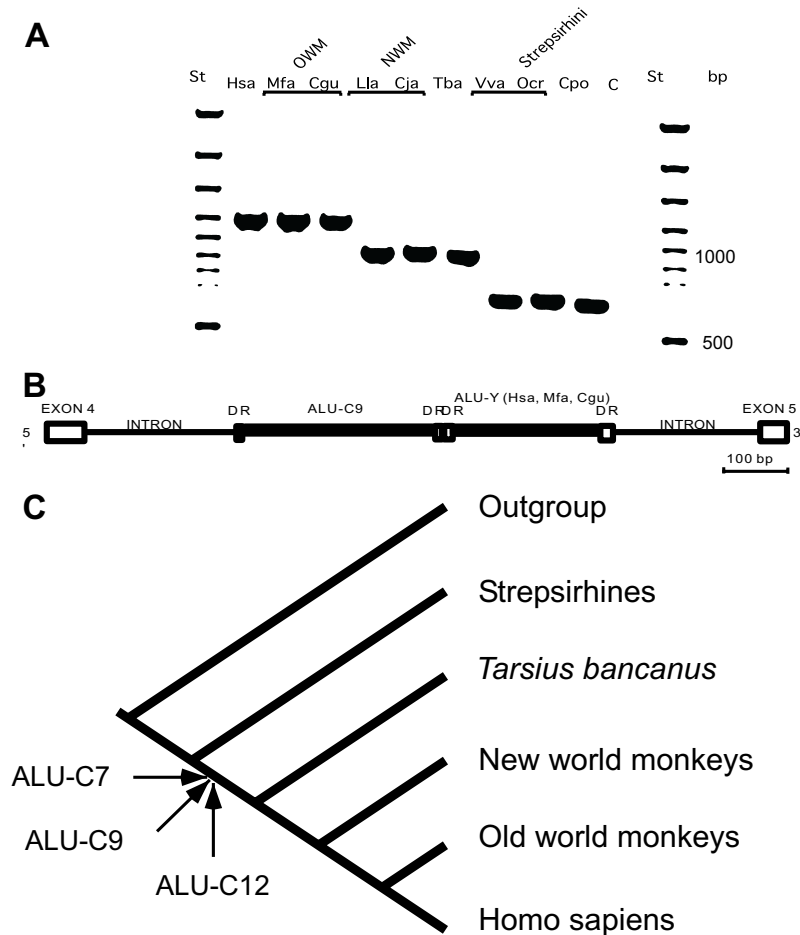
The reason for choosing the mitochondrial genome as a basis for our phylogenetic reconstructions is mainly due to the fact that a taxonomically broad published data set on complete mitochondrial genomes exists, in which the Tupaia sequence could be incorporated. However it has to be noted, that the presumably short branching intervals and the long independent evolutionary histories of the terminal taxa, that can be observed in mammalian evolution, represent a major difficulty with respect to the phylogenetic reconstruction on the basis of even complete mitochondrial genomes. Thus, to corroborate the above mentioned hypothesis, further analyses are still mandatory, which beside mitochondrial sequence information should incorporate morphological data, and nuclear sequence analyses as well as a systematic analysis of synapomorphic-SINE insertions. Concerning the latter, we have started a project in which the phylogenetic affiliations of the Scandentia to Lagomorpha and other eutherian orders will be analysed by the shared presence of SINEs at orthologous loci.

#### *Tarsius as a sistergroup to the Anthroipoidea*

In the report year nuclear transpositions, mainly transpositions of Alu-elements were used as molecular-cladistic markers. By querying the database, intronic Alu-elements were defined, and their distribution in different primate taxa determined by PCR with exonic, conserved primers. The primary goal was to clarify the primate infraordinal relationships i.e. to corroborate the pattern of successive branchings that took place on the lineage to humans with molecular-cladistic arguments. In this context, the most important question pertains to a clear definition of the sistergroup to the Anthroipoidea. Whereas neontological, morphological data suggest a sistergroup affiliation of the infraorder of tarsiiiformes to the Anthroipoidea (Old World monkeys, hominoids and New World monkeys), the incorporation of palaeontological data suggests other possible sistergroup relations of tarsiiiformes e.g. with strepsirrhines (lemuriformes and lorisiformes). This discrepancy is in part due to the fact that *Tarsius* is the only extant representative of a formerly diverse group of Eocene

tarsiiformes. It is therefore conceivable, that not all characters traceable in fossils can be completely represented by the extant *Tarsius* species. Moreover, *Tarsius* could accumulate numerous autapomorphies in its long independent evolutionary history. The molecular analyses carried out to date have not been able to adequately solve this discrepancy. Whereas nuclear DNA analyses have not been able to resolve the *Tarsius* position with satisfying significance, the incorporation of mtDNA data give rise to an even more puzzling picture. MtDNA comparisons of *Tarsius* sequences with orthologues from other primates and mammals place *Tarsius* \pard fl apart from the primates, which, according to the proposals of the Easteal group (1998) might be caused by a deviation of the mtDNA sequence evolution from a purely neutral mode of evolution. We, therefore, chose to tackle this question using an alternative strategy and applying a molecular cladistic approach. Thus, transpositions of Alu-sequences, representing the most abundant SINE family in primate genomes, were used to analyse the infraordinal relationships in primates. Altogether 118 loci that contained intronic Alu-elements in humans were PCR-analysed, in each of two representatives of the lemuriformes/lorisiformes, the New World monkeys, Old World monkeys, *Tarsius bancanus* and a non-primate outgroup, to detect the presence of orthologous Alu-elements in the respective taxa. 14 of these markers displayed a fragment-length pattern, which showed higher molecular weight bands for all anthropoid representatives and *Tarsius*, whereas shorter fragments could be detected in the non-primate outgroup and the strepsirrhine representatives. From these, subsequent sequence analyses revealed three Alu transpositions, which represent shared derived characters in *Tarsius* and all anthropoid taxa tested. The ancestral character state, which is the occurrence of an unoccupied transposition target site, was determined at the orthologous sites both in the strepsirrhine representatives and the non-primate outgroups. This confirms, that the three Alu-elements seen at orthologous positions in *Tarsius* and the Anthroidea arose in a common ancestor and, thus, ensure their sistergroup relationship.

The fact that only three out of 118 tested markers support this phylogenetic relationship could be explained for example by a reduced transpositional activity of Alu-elements on the lineage leading to the most recent common ancestor of *Tarsius* and the Anthroidea. However, conventional phylogenetic sequence analyses carried out in parallel show, that the consecutive splittings to the strepsirrhines and to *Tarsius* have taken place in a comparatively short time interval. Regarding the other 11 markers that were analysed on the sequence level, molecular scenarios were deduced involving the transpositions of unrelated SINEs or suggesting independent transpositions of Alu-elements on different evolutionary lineages leading to extant *Tarsius* and on the lineage leading to the most recent common ancestor of the Anthroidea. The latter took place in the same intron, albeit at different positions. In one case we detected the presence of two independent Alu-transpositions that took place merely 40 bp apart from each other. The fact that two molecular integration events that date back several dozens of millions of years and which are physically separated by only 40 bp could be unequivocally discriminated demonstrates the strength of this molecular cladistic approach. Moreover, the identification of transposition target sites in the non-primate outgroups, represented by mammalian taxa that share a common ancestor with primates in the range of 100 MYA (million years ago) suggests that this approach can be applied to analyse even deeper splits, e.g. the



*A: Amplification pattern at the locus C9 as obtained from Cavia porcellus (Cpo), Otolemur crassicaudatus (Ocr), Varecia variegata (Vva), Tarsius bancanus (Tba), Callithrix jacchus (Cja), Lagothrix lagothricha (Lla), Colobus guereza (Cgu), Macaca fascicularis (Mfa) and humans (Hsa). The pattern can be explained by two consecutive integrations of Alu-elements taking place on the lineage to the most recent common ancestor of Tarsius and all Anthropoids and on the lineage to the most recent common ancestor of Old World monkeys and hominoids. The absence of integration representing the ancestral character state at this locus is reflected by the amplification product with the smallest fragment length as observed for the representatives of the Lemuriformes/Lorisiformes and the outgroup (guinea pig).*

*B: Schematic representation of the two Alu-elements as observed in humans. The exon-intron structure at this locus is depicted as well as the direct repeats at both ends of the transposed DNA which originate during integration. The 3' Alu-element belongs to the Alu-Y-family and can only be traced in Old World monkeys/Hominoids at this locus.*

*C: Schematic representation of the phylogenetic affiliations of the primate infraorders and humans. Arrows indicate the transpositional events that support the sistergroup relationship of Tarsius and the Anthroidea.*

origin of primates (see also the above mentioned problematics regarding the phylogeny of Scandentia). However, it has to be noted, that the occurrence of highly repetitive Alu-elements is restricted to the order of primates. Thus an effective approach to analyse the primate interordinal relationships to other mammals can be only carried out by exploiting the information of the complete human and mouse sequences and analysing other types of SINEs (e.g. MIR sequences). This however requires intensive future research on the time periods of transpositional activity of certain SINEs during mammalian evolution as well as on defining the respective integration preferences on the sequence level.

*Transpositional markers in New World monkeys and strepsirhines*

In analogy to the Tarsius problem, SINE information is used to determine branching patterns in New World monkeys and strepsirhines. Several markers informative for some critical branching events in the phylogeny of callitrichid monkeys were established during the report year. Concerning the strepsirhines, the Alu-information obtained to date is currently being complemented by the characterisation of tRNA-derived SINEs. In this context, several tRNA-derived elements which were obtained after database searches as well as isolated from enriched genomic libraries constructed from *Lori tardigradus*- and *Perodicticus potto*-DNA have been characterised. These markers are currently being analysed with respect to their taxonomic distribution.

*Classical sequence analyses*

Beside the molecular cladistic approach, mitochondrial sequences of representatives of several primate infraorders e.g. colobine monkeys (cooperation Nadler), strepsirhines, gibbons (cooperation Geissmann) and Tarsius were obtained. These sequences are currently being used to estimate the respective branching dates and are, complementary to the cladistic approach, used to analyse questionable phylogenetic tree topologies in the respective groups of taxa. Concerning the gibbons, sequences of the mitochondrial control region together with the adjacent PHE-tRNA of the four extant gibbon subgenera were described. In contrast to previous studies on gibbon phylogeny, which were based on other mtDNA segments, it could be shown, that the *Nomascus* group represents the most basal group of the hylobatids, followed by *Symphalangus* and *Bunopithecus*, whereas *Hylobates* constitutes the most recently diverging taxon. Moreover these data show, that the genetic distances as obtained for the four gibbon subgenera are in the same range or even higher as compared to the distances between humans and chimpanzees. Thus, a possible consequence of this observation could be to attribute a taxonomic status of a genus to them.

**Population genetics**

During the report year the establishment and characterisation of molecular marker systems for Old World monkeys, New World monkeys and strepsirhines was continued and partially applied in practice in co-operation with scientists working in the field. Both nuclear microsatellites and hypervariable regions of mitochondrial DNA were defined and applied as infraspecific, molecular marker systems. With re-

spect to nuclear, tandem repetitive markers, two strategies were applied for their establishment.

Concerning the New World monkey genus *Saguinus* (cooperation Heymann), microsatellites were isolated *de novo*, whereas the approach of trans-species applications of microsatellites was intensified for the strepsirhine infraorder. During the report year microsatellite loci were systematically transferred from *Cheirogaleus medius* (cooperation Fietz/Ganzhorn) to the two other Cheirogaleid genera *Phaner* and *Microcebus* (cooperation Eberle/Ganzhorn/Kappeler/Schülke). One aim of these studies is to reveal reproductive strategies of these animals. Cheirogaleids belong, as descendants of the deepest split of the primate order, to its most primitive representatives. Their social system, therefore, has been considered to reflect an evolutionary ancestral state in primates. The first genetic data obtained from free ranging *Cheirogaleus medius* were published during the report year. Accepted models of social evolution in primates state that pair-bonding has evolved secondarily from diurnal group-living taxa. Pair-bonding should, therefore, be constrained primarily to diurnal species. Contrary to these assumptions, the nocturnal *Cheirogaleus medius* lives in permanent pairs. Combined field observations and genetic studies on the kinship among individuals of social groups showed that yearlings and infants were, in all cases, offspring of the social mother, which does not hold for the paternal site. 44 % of the infants were not sired by the social father, rather representing offspring of male individuals living in other pair-bonds. In this way, males may increase their reproductive success, females on the other hand do not seem to run the risk of reduced paternal care during the raising of the offspring, because the social fathers cannot detect relatedness of the respective young. In contrast to territory holders, floating males could not sire offspring.

During the report year, work on *Cheirogaleus* was extended with the aim to set up an exhaustive analysis of the population genetics, phylogeography and the possible influences of ecological factors for this taxon. For this, further microsatellites were characterised together with the mitochondrial control region of *Cheirogaleus*, to estimate migration and gene flow between local populations living under different ecological conditions in a gender-contrasting manner. Furthermore, by comparing the D-loop information of individuals originating from different geographic locations, the genetic distance between local populations will be determined and compared to genetic distances of other strepsirhines e.g. *Microcebus*. Our aim is to mainly compare the morphological and the genetic variability, to draw conclusions about the species/subspecies status of these taxon groups based on the phylogenetic species principle. Parallel to molecular labwork, the respective fieldwork was started during the report year.

In this context the question of the still debated phylogeny and systematics of the genus *Pygathrix* was addressed during the report year (cooperation Nadler). The limited knowledge about this taxon is mainly due to the unavailability of the sample material, since Douc langurs are both extremely rare in the wild, as well as under-represented such zoos or museum collections. In the year under report, the first comprehensive molecular analysis in which 576 bp of the mitochondrial cytochrome *b* gene of three extant Douc langur species were sequenced was finished. The data can be interpreted such that *P. nigripes* represents the deepest split, whereas *P. cinerea* and *P. nemaus* show a sistergroup relationship to each other. On the basis of these

data, all three taxa could be regarded as separate species according to the phylogenetic species principle. Moreover, two deep splits could be detected in the *P. nemaues* as well as in the *P. nigripes*-clade, which represent geographic variation or even a subspecies-status for the respective taxa groups.

#### *Evolution of tandem repetitive DNA*

In the report year, analysis of the evolution of tandem repetitive DNA was started thus going beyond the pure application of tandem repetitive loci as marker systems. As a prerequisite for the interpretation of genetic data obtained from variable loci, the exact underlying evolutionary mechanisms need to be known. In the case of variability generation at microsatellite loci, the main basis for (length) polymorphisms is assumed to be caused by replication slippage whereas point mutations are considered to play a minor role. The dynamics of the evolution of microsatellite loci were mainly elucidated from comparative infraspecific analyses or from analysing different individuals belonging to closely related species. A phylogenetic perspective on the evolution of short tandem repeats could yield precious information, however comparatively little work has been done on primates regarding this topic. In the report year, we worked on the analysis of the origin and evolution of the microsatellites initially described for *Saguinus* and *Cheirogaleus* along different lineages in the order of primates. The primers constructed for the respective taxa amplified homologous loci in several other taxa (see above for e.g. *Phaner*, *Microcebus*), thus, the origin, extension and a possible internal structure and mutational polarity of the repetitive unit could be traced. Concerning the microsatellites originally developed for paternity tests in *Saguinus mystax*, five primer pairs were cross-species applied and the respective fragments sequenced in different taxa representing the New World monkeys as well as Old World monkeys and hominoids. In addition several markers were applied in small population surveys involving 10 – 30 individuals from *Cebus apella*, *Saimiri sciureus* and *Callithrix jacchus*, to check for infraspecific length variability. All microsatellites were traced in all species analysed, however with increasing phylogenetic distance they were found to become more rudimentary. It seems that point mutations interrupting a perfect microsatellite motif-reiteration play a key role in such a “death” of a microsatellite. Point mutations interrupt longer perfect motif reiterations, which are in consequence less prone to slippage and thus might disappear. In contrast the infraspecific variability is mainly due to repeat length polymorphisms, where it could be especially observed in composite microsatellites, that reiterations of more than ten motifs are prone to repeat expansion. Although only loci that can be successfully amplified across species with a considerable phylogenetic distance, e.g. New World monkeys and humans, and therefore exhibit at least conserved primer sites were analysed, it could be shown, that the repeat flanking regions exhibit a considerable degree of polymorphism, possibly rendering them useful for phylogenetic analyses. In particular, informative length polymorphisms (insertions/deletions) could be observed in the repeat flanks beside point mutations.

Further analyses pertaining to the evolution of tandem repetitive DNA were carried out on presumably neutrally evolving triplet-loci and for a protein coding locus represented by the mucin domain of the zonadhesin gene. On the basis of the DHGP cosmid libraries established during the last year for *Callithrix jacchus*, *Macaca*

*mulatta* and *Tupaia belangeri*, we tried to find the respective orthologues of four triplet loci initially described in humans. However, we did not succeed for any of the loci, which might be possibly explained by an underrepresentation of repetitive sequences in the library, which are assumed to be unstable during the cloning process. The zonadhesin repeat examinations are still running, with an emphasis on the analysis of the germ line variability (mutation rate) which is carried out by small pool PCRs of both blood as well as sperm derived DNA.

### **Immunogenetics**

The term MHC molecule describes two classes of membrane-anchored glycoproteins which present peptide antigens to the T cells of the immune system. The highly polymorphic genes of the Major Histocompatibility Complex (MHC) play an important role in triggering and regulating the immune response. The recognition of a peptide bound to the MHC protein activates antigen-presenting cells and T cells, the release of messenger substances and finally an immune response. Since every MHC protein is only able to present a specific set of peptides, different immune responses can be triggered in an individual depending on their MHC make up. Since the MHC genes play such an important role in the immune system, many immunological disorders as well as the resistance or susceptibility to infectious diseases have been linked with the expression of particular MHC molecules.

The working group Immunogenetics at the DPZ primarily focuses on investigating the genetic structure of the rhesus monkey (*Macaca mulatta*) MHC and the development of fast typing methods. The main goal is to identify MHC alleles associated with disease progression of AIDS in macaques. Another goal is to elucidate whether protection by vaccination is associated with specific MHC molecules. Therefore, the immunogenetics group has been typing experimental animals for a national AIDS Research network for years. Furthermore we have taken over the central typing of experimental animals for a European research network and a US vaccine study.

Despite their minor role in triggering an immune response in comparison with the DRB genes, our focus of the past years has been on the MHC class II encoded DQ-genes of the rhesus monkey. Because of the lower complexity of DQ genes, typing methods were developed faster than would have been possible for DRB genes. Since DQ genes are tightly linked to the DRB genes in our experimental animals, one aim was to use DQ-typed DNA samples to specifically describe DRB haplotypes. In addition, we expected an at least weak association between disease progression of AIDS and the DQ genes.

According to the results obtained so far, this assumption proved to be correct. The characterisation of 10 common DQ-DRB haplotypes in 3 rhesus monkey populations showed the presence of considerably more DRB haplotypes in rhesus monkeys as compared to humans. The allelic polymorphism of a defined DQ-DRB haplotype is lower in rhesus monkeys than in humans. In the rhesus monkey populations examined to date, the linkage between DQ and DRB genes turned out to be tighter in rhesus monkeys than in humans. Hence, more precise methods for typing rhesus monkeys DQB-genes were developed in the Immunogenetics group during the last year. This resulted in the discovery of 10 novel DQB-alleles, thus the sequences of 38 rhe-

sus monkey DQB alleles (compared to 46 in human) are known to date. At least 22 novel DRB variants were detected by a sequence-based typing of DRB alleles from laboratory animals. Meanwhile, far more than 100 DRB alleles of rhesus macaques have been published. All data at hand allow the interpretation that the MHC in rhesus monkeys is substantially more complex than in humans. Another focus of the immunogenetics group is the characterisation of MHC class I genes of rhesus monkeys. As a part of his doctoral thesis Thorsten Mühl was able to develop a method for identifying and sequencing MHC class I genes using RT-PCR and denaturing gradient gel electrophoresis. In this way he was able to identify a set of novel MHC class I alleles. In order to develop sequence-based screening methods, 14 primer pairs were designed. By sequencing the respective PCR-products it is possible to discriminate between 20 class I alleles.

After typing of about 90 non-immunised SIV-infected monkeys, alleles that are associated with slow disease progression were determined.

MHC class II genotypes that are associated with rapid disease progression have already been described by us. By means of an analysis of both the MHC class I and class II variants, the disease progression of SIV-infected animals can now be relatively exactly predicted, albeit dependent on the origin of the animals.

Therefore, we now identify the animals with predictable biological characteristics prior to the respective experiments. Such animals are particularly suited to investigate immunological and virological parameters which might influence different disease progressions in SIV-infected monkeys. The respective investigations were carried out in cooperation with Dr. Stahl-Hennig and Dr. Spring from the Department of Virology and Immunology. At present however, a problem arises in that sufficient numbers of rhesus monkeys cannot be bred neither at the DPZ nor in Europe. The laboratory animals, therefore, have to be obtained from changing sources. At present, monkeys are imported possessing MHC-alleles that are, in part, completely different from the alleles of the rhesus monkeys analysed earlier. Alleles, for which their influence on the disease progression is already known, exhibit an only low abundance in this sample of animals. These animals also appear to react differently to SIV-infections. This shows the importance of inclusion only of animals that were obtained from a defined breeding program or alternatively from a breeding program set up by ourselves, in the experiments.

Within an EU study, the development of MHC typing of long-tailed macaques (*Macaca fascicularis*) was started. To date, typing methods for the DRB genes of long-tailed macaques originating from Mauritius were set up. In these monkeys, that are closely related to each other, 11 DRB alleles were detected, to date, for which fast sequencing-based screening methods have been established. By applying the same methods, the laboratory animals within a European BSE-project can also be tested to address the question of an immunogenetical predisposition to various degrees of susceptibility to BSE infections.

Within the "Graduiertenkolleg: Perspectives of Primatology", methods for typing the DRB genes of lion-tailed macaques (*Macaca silenus*) from the DPZ and from zoos have been developed in cooperation with the Department of Veterinary Medicine and Primate Husbandry. This research is being carried out under the aspect of a possible genetic basis related to the susceptibility to echinococcosis, a lethal infection of lion-tailed macaques.

**Projects and partners in cooperation**

(I: interdepartmental project, E: external cooperation, A: completed project, L: current project)

<b>Projects and Partners of the Working Group Primate Genetics</b>		
<p><b>Relevance of MHC class I and class II alleles for immune pathogenesis of attenuated SIV in rhesus monkeys (<i>Macaca mulatta</i>)</b></p> <p>U. SAUERMAN, T. MÜHL, C. STAHL-HENNIG, M. SPRING, N. STOLTE (Dept. of Virology and Immunology, DPZ), S. SOPPER (Inst. of Virology and Immunobiology, Univ. Würzburg), K. ÜBERLA (Inst. of Virology, Leipzig)</p>	E,I	L
<p><b>Do MHC genes influence reproductive behaviour or reproductive success in rhesus monkeys?</b></p> <p>U. SAUERMAN, P. NÜRNBERG (Inst. of Medical Genetics, Humboldt-Univ. Berlin), J. SCHMIDTKE (School of Medicine, Inst. of Human Genetics, Hannover), F. BERCOVITCH, J. BERARD (Caribbean Primate Center, Puerto Rico, USA), M. KRAWCZAK (Univ. Wales, Inst. of Medical Genetics, GB)</p>	E	L
<p><b>Characterisation and expression of rhesus monkey MHC class I variants</b></p> <p>T. MÜHL, U. SAUERMAN, A. STUKE, H. PETRY (Dept. of Virology and Immunology, DPZ)</p>	I	L
<p><b>Association of MHC-genes with disease progression and protection by vaccination in SIV-infected monkeys</b></p> <p>U. SAUERMAN, E. KRAISELBURD (Caribbean Primate Center, Puerto Rico, USA)</p>	E	L
<p><b>Central typing of rhesus and long-tailed monkeys within an EU project on vaccination experiments</b></p> <p>U. SAUERMAN, C. STAHL-HENNIG, G. HUNSMANN (Dept. of Virology and Immunology, DPZ), F. TITTI (ISS, Rom, I), S. NORLEY (RKI, Berlin), M. CRANAGE (CAMR, Salisbury, GB), N. ALMOND (NICBS, Hertfordshire, GB), R. LEGRAND (Centre de Recherches, Fontenay-aux-Roses, F), G. BIBERFELD (SIIDC, Lundagatan, S), J. HEENEY (BPRC, Rijswijk, NL)</p>	E,I	L
<p><b>Morphological, serological and genetic investigations on spontaneous echinococcosis in a colony of lion-tailed macaques (<i>Macaca silenus</i>)</b></p> <p>A. BLANKENBURG, U. SAUERMAN, S. RENSING, K. MÄTZ-RENSING, F.-J. KAUP (Dept. of Veterinary Medicine and Primate Husbandry, DPZ)</p>	I	L

*Working Group Primate Genetics*

<b>Projects and Partners of the Working Group Primate Genetics</b>		
<b>MHC-typing of long-tailed macaques within a European BSE-project</b> U. HAHMANN, A. STUKE (Dept. of Virology and Immunology, DPZ), <b>U. SAUERMANN</b>	I	L
<b>Conservation biology of Malagasy lemurs</b> B. RAKOTOSAMIMANANA, Univ. Antananarivo, Madagascar), J. GANZHORN (Inst. of Zoology, Hamburg Univ.), S. GOODMANN (FMNH Chicago & WWF Madagascar), K. HODGES (Dept. of Reproductive Biology, DPZ), <b>H. ZISCHLER</b> , P. KAPPELER (Dept. of Ethology and Ecology, DPZ)	E,I	L
<b>Population genetics in Eritrean hamadryas baboons</b> <b>A. HAPKE</b> , <b>H. ZISCHLER</b> , D. ZINNER (Dept. of Ethology and Ecology, DPZ)	I	L
<b>Phylogeny of New World Monkeys</b> <b>S. SINGER</b> , <b>J. SCHMITZ</b> , <b>H. ZISCHLER</b> , E.W. HEYMANN (Dept. of Ethology and Ecology, DPZ)	I	L
<b>Proximate regulation and genetic consequences of the mating system in polyandrous tamarins</b> M. HEISTERMANN, K. HODGES (Dept. of Reproductive Biology, DPZ), <b>U-R. BÖHLE</b> , <b>C. SCHWIEGK</b> , E.W. HEYMANN (Dept. of Ethology and Ecology, DPZ)	I	L
<b>Population genetics and - differentiation of <i>Cheirogaleus medius</i></b> <b>A. HAPKE</b> , <b>H. ZISCHLER</b> , J. GANZHORN (Inst. of Zoology, Hamburg Univ.)	E	L
<b>Phylogeny of Colobinae and Hylobatidae</b> <b>C. ROOS</b> , T. GEISSMANN (School of Veterinary Medicine, Hannover), T. NADLER (EPRC, Vietnam)	E	L
<b>Genetic analysis of the mating system in polygynous lemurs</b> B. WIMMER, D. TAUTZ (Köln Univ.), <b>H. ZISCHLER</b> , M. EBERLE, P. KAPPELER (Dept. of Ethology and Ecology, DPZ)	E,I	L
<b>Echinococcosis in semi-free ranging non-human primates</b> <b>A. BLANKENBURG</b> , K. MÄTZ-RENSING, S. RENSING (Dept. of Veterinary Medicine and Primate Husbandry), K. JANITSCHKE (RKI, Berlin), <b>U. SAUERMANN</b> , F.J. KAUP (Dept. of Veterinary Medicine and Primate Husbandry, DPZ)	E,I	L

**Scientific contributions**

**Diploma theses**

FISCHER, A.: Investigations on the copy number ratio of nucDNA and mtDNA in different human tissues. Biological Faculty, FU Berlin (2000).

**Congress contributions**

Annual meeting of the German Society of Tropical Ecology (gtö), Würzburg, 01.-05.03.00, ZINNER, D., HAPKE, A.: Ecological comparison of primates in Eritrea.

5<sup>th</sup> European conference on Experimental AIDS Research, Madrid, E, 16.-19.06.00, MÜHL, T., STAHL-HENNIG, C., SAUERMANN, U.: Identification of MHC class-I alleles associated with disease progression in SIV-infected macaques.

18<sup>th</sup> Annual Symposium on Nonhuman Primate Models for AIDS, Madison, USA, 03.-08.10.00, SAUERMANN, U., STAHL-HENNIG, C., MÜHL, T., STOLTE, N., KAUP, F.-J., ÜBERLA, K., HUNSMANN, G., SOPPER, S., SPRING, M.: AIDS-related disease in macaques infected with attenuated SIV is mediated by host factors.

**Seminar lectures**

Seminar of the Medical Genetics Department of the LMU, München. 08.02.00, ZISCHLER, H.: Nuclear integrations of mitochondrial DNA and SINE-transpositions as phylogenetic markers in primates.

Seminar of the SFB 399 "Molecular pathology of proliferation", University of Homburg, 14.03.00, ZISCHLER, H.: Nuclear integrations of mitochondrial DNA and SINE-transpositions as phylogenetic markers in primates.

Seminar of the Institute of Human Genetics and Anthropology of the University of Freiburg, 19.07.00, ZISCHLER, H.: Molecular cladistic markers in primate phylogeny.

Meeting of the "Forschungsverbund Göttingen-Würzburg, SIV-Infektion von Rhesus-Makaken und Vakzinierungsansätze", Göttingen, 27.11.00, SAUERMANN, U.: The role of MHC polymorphisms in SIV-infections.

**Other scientific activities**

- **Dr. Sauer mann** is an associated member of the Editorial Board (associate editor) of *Current Molecular Medicine*