

lined with a diverse microbial flora (Fig. 1F). We attempted to confirm the absence of microorganisms in the digestive tracts of *Limnoria* spp. by using conventional microbiological plating techniques. However, the small size of the gut and the extensive microbial colonization of the complex exoskeleton surfaces (Fig. 1E) prevent use of these methods.

Bacteriological studies of the decapod *Homarus vulgaris* (11) and the deep-sea scavenging amphipod *Hirondellea* sp. (12) recently demonstrated that these crustaceans maintain an indigenous microflora within the stomach and intestine. The mechanisms by which the crustaceans observed in our study maintain a bacteria-free digestive system remain unexplained. It is possible that enzymes produced by these organisms lyse ingested bacteria (13). However, no bacterial cell fragments have been observed within the gut. A gut exudate may be toxic to bacteria. This hypothesis is supported by our observation that *L. tripunctata* fecal matter is not readily colonized by bacteria. In contrast, the overlying water supports an abundant microflora. Further research into the antimicrobial processes active in these crustaceans is needed.

The precise microbiological status of the gastrointestinal systems in the overwhelming majority of invertebrates remains to be determined (14). Marine wood-boring bivalves and oysters are known to possess an indigenous microflora in their intestinal tracts (15). Echinoids have also been found to support a gut microflora (16). However, the species of *Limnoria*, *Chelura*, and *Oniscus* observed in our laboratory appear to be the only known metazoans with digestive systems naturally free of microorganisms. The bacteria-free gut, observed as a normal condition in these invertebrates, should provide a model system for research on axenic and gnotobiotic digestive tracts, which has heretofore depended on extraordinary measures to produce artificial sterility. Furthermore, it may provide a new source of antimicrobial agents.

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9. Fixation was in 2 percent glutaraldehyde buffered with 0.025M sodium cacodylate in natural seawater filtered at 0.45 μ m. Dehydration was accomplished in an acetone-distilled water series (10, 20, 40, 75, 95, and 100 percent acetone). Critical point drying was in liquid CO₂; samples were viewed under an AMR 1000 scanning electron microscope at the Harvard Museum of Comparative Zoology.
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17. Supported by grant N00014-76-C-0042, NR-104-967 from the Office of Naval Research. We thank H. Ducklow and R. Sjoblad for critical reviews of the manuscript and for their interest and suggestions during the research. We also thank R. Turner for many valuable discussions, W. Kaplan for reviewing the manuscript, E. Selig for expert technical assistance with the scanning electron microscope, and R. Turner, C. Berg, D. Bultman, and B. Boyle for help in obtaining specimens.

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Amino Acid Sequence of the Fc Region of a Canine Immunoglobulin M: Interspecies Homology for the IgM Class

Abstract. *The amino acid structure for the Fc portion of a canine immunoglobulin μ chain was determined. The sequence was compared with those of two human μ chains, and a high degree of interspecies homology was observed. The preservation of primary structure between species is probably reflective of the unique functions associated with the immunoglobulin M class.*

Amino acid sequence analyses of the κ , λ , and γ chain constant regions of immunoglobulin reveal a modest degree of sequence homology when these regions are compared with respect to species (1–3). When the amino acid residues at a particular position in immunoglobulin chains are compared, most studies indicate approximately 60 percent preservation of primary structure (4). In the variable region, such calculations are more complicated because of V region subgroups. However, where subgroups can be clearly related, the interspecies homology within the variable region also approximates 60 percent. If hypervariable regions are excluded from this comparison, homology in the variable region often approaches 85 to 90 percent (5, 6).

Phylogenetic, evolutionary, and immunochemical data suggest that the immunoglobulin M (IgM) class has been more rigidly preserved in evolution than κ , λ , or γ chains (7). Studies on the serologic cross-reactivity of the various classes and types of immunoglobulins have repeatedly demonstrated that IgM molecules cross react more extensively

than do immunoglobulin G (IgG), immunoglobulin A (IgA), κ , or λ chains (8).

We have recently completed the variable region sequences of a canine IgA and a canine IgM (9). We now report the complete amino acid sequence of the Fc region of a canine μ chain and compare the sequence with human μ chains.

The monoclonal canine IgM Moo was isolated by plasmapheresis from the serum of a dog with lymphosarcoma. Starch block electrophoresis, ion exchange chromatography, and chain separation techniques were used to determine its primary structure (9). Cyanogen bromide peptides were isolated and subjected to extensive amino terminal sequence analysis with an automated Beckman 890C sequencer. Subsequently, appropriate tryptic peptides were isolated and sequenced, some with the aid of Polybrene (10). With only a few exceptions, the entire Fc region of the molecule has been sequenced and extensive overlaps were obtained.

Figure 1 presents the amino acid sequence of 272 residues of the Moo Fc region compared to the human Ou (11) and Gal (12) μ chains. The Fc regions of Ou

and Gal differ from each other by nine amino acids excluding acid or amide assignments (amino acid residues 314, 342, 373, 386 to 388, 418 to 419, 488a). A straight line in the figure indicates identity with the Moo sequence. An insertion (amino acid residue 488a) and a deletion (amino acid residue 373) have been introduced in the human μ chains to obtain maximum homology; however, neither insertion nor deletion was required to align the canine and human sequences.

As judged by a comparison of the entire Fc regions (13), the homology of the canine μ chain with the human μ chains is greater than 82 percent. This high degree of homology is more evident when the functionally separate portions of the Fc region are considered. For example, the 68 percent homology in the hinge region (amino acid residues 305 to 350), while less than that seen overall, is striking since this region is extraordinarily variable, even among subclasses of human and guinea pig IgG. The third constant region domain (amino acid residues 351 to 450) and the fourth constant re-

gion domain (amino acid residues 451 to 576) show 86 and 85 percent homology, respectively. From positions 500 to 576, there are only six amino acid differences, representing 92 percent homology. Thus, there appears to be a gradient of increasing homology from the hinge region to the carboxy-terminus.

The regions of maximum difference between the canine and human μ chains occur at the inter-heavy (H) chain disulfide bond at position 337, with only four identities in the next 12 positions, and at the disulfide bond at position 414, tentatively assigned as the intersubunit bond, where from position 412 to 417 there are only two identities. While the exact assignments of the inter-H chain disulfide bridges at positions 337, 414, and 575 as intra- or intersubunit remains uncertain, these data suggest that the structural constraints on position 575 are different and more stringent than on positions 337 and 414. With the exception of these two disulfide bond regions, the comparison of the entire Fc position shows a greater than 95 percent structural homology between molecules derived from two dis-

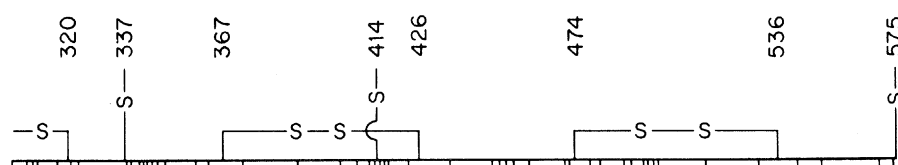
tinct species belonging to separate mammalian orders.

The interspecies amino acid sequence homology of the Fc portion of the μ chain compared to γ , κ , and λ chains probably reflects the different functions associated with the IgM class. The structural requirements for the major B cell receptor may preclude the kinds of amino acid interchanges that have occurred in the IgG molecule. In addition, the requirement for polymer formation in IgM may exert a strong selection pressure against many mutational events in the carboxyl-terminal region of the IgM molecule. Structural comparisons of α chains from different species may be informative in this regard, since the carboxyl-terminal regions of human IgA and IgM are remarkably similar (14), despite the significant differences between their Fd domains (11, 12). Whatever the mechanisms responsible for preserving structure in the Fc region between canine and human μ chains, our study demonstrates that interspecies homologies in immunoglobulins must be evaluated from the standpoint of immuno-

Fig. 1 (top). The amino acid sequence of the Moo canine IgM Fc is shown above and compared to those of Ou and Gal. The sequences are numbered according to Ou numbering. One deletion (373) and one insertion (488a) in the Gal protein is necessary to align the human proteins. A straight line indicates identity with the Moo sequence. The hinge region, third constant domain, and fourth constant domain have been arbitrarily defined as amino acid residues 305 to 350, 351 to 450, and 451 to 576, respectively. One-letter abbreviations for the amino acid residues are as follows: alanine, A; aspartic acid or asparagine, B; cysteine, C; aspartic acid, D; glutamic acid, E; phenylalanine, F; glycine, G; histidine, H; isoleucine, I; lysine, K; leucine, L; methionine, M; asparagine, N; proline, P; glutamine, Q; arginine, R; serine, S; threonine, T; valine, V; tryptophan, W; unknown or other, X; tyrosine, Y; glutamic acid or glutamine, Z.

	310	320	330	340	350
Moo	M L T I Q E D A W L S Q S V F T C K V E H R G L T F Q Q N A S S M C T S D Q P V G I S I F T I				
Ou	T _ _ _ K Z S D _ _ G E _ M _ _ _ _ _ R _ D _ _ _ _ _ _ _ _ _ _ _ _ _ _ V P _ _ D T A _ R V _ A _				
Gal	T _ _ _ K _ S B _ _ _ _ _ M _ _ _ _ _ R _ D _ _ _ _ _ B _ _ _ _ _ V P _ _ Z R T A _ R V _ A _				
	360	370	380	390	400
Moo	P P S F A S I F N T K S A K L S C L V T D L A T Y D S V T I S W T R E E N G A L K T H T N I S E S H				
Ou	_ _ _ _ _ L _ _ _ T _ _ T _ _ _ _ _ T _ _ B _ _ _ _ _ _ _ _ _ _ V _ _ _ _ _				
Gal	_ _ _ _ _ L _ _ _ T _ _ T _ _ _ _ _ () _ _ _ _ _ Q D G E _ V _ _ _ _ _				
	410	420	430	440	450
Moo	P N G T F S A M G E A T V C V E E W E S G E Q F T C T V T H T D L P S V L K Q T I S R P K G V A V H				
Ou	_ A _ _ _ V _ _ _ S I _ E D B D W _ _ _ R _ _ _ _ _ P _ _ _ _ _ L _ _				
Gal	_ B A _ _ _ V _ _ _ S I _ E B B _ B _ _ _ R _ _ _ _ _ P _ _ _ _ _ L _ _				
	460	470	480	490	500
Moo	M P S V Y V L P P S R E Q L D L R E S A T L S C L V T G Y S P P D V F V Q W V Q K G Q P V P P D S Y				
Ou	R _ B _ _ L _ _ _ A _ Z Z _ N _ _ _ _ _ I T _ _ _ _ _ F _ _ A _ _ _ E _ M _ R _ E _ L S _ Q K _				
Gal	R _ D _ _ L _ _ _ A _ _ _ _ N _ _ _ _ _ I T _ _ _ _ _ F _ _ A _ _ _ Q M _ R _ _ _ L S _ E K _				
	510	520	530	540	550
Moo	V T S A P M P E P Q A P G L Y F A H S I L T V S E E E W N A G E T Y T C V V A H E S L P N R V T E R				
Ou	_ _ _ _ _ _ _ _ _ _ _ R _ _ _ _ _ _ _ _ _ _ T _ Q _ _ _ _ _ A _ _ _ _ _				
Gal	_ _ _ _ _ _ _ _ _ _ _ R _ _ _ _ _ _ _ _ _ _ T _ _ _ _ _ _ _ _ _ _ A _ _ _ _ _				
	560	570			
Moo	S V D K S T G K (P T L Y) N V S L V L S D T A G Z C Y				
Ou	T _ _ _ _ _ _ _ _ _ M _ _ _ _ _ T _ _ _ _ _				
Gal	T _ _ _ _ _ _ _ _ _ B _ _ _ _ _ M _ _ _ _ _ T _ _ _ _ _				

Fig. 2 (bottom). Schematic diagram illustrating the location of the sequence differences between canine and human μ chains. Differences have been illustrated by a vertical line below the horizontal line. The disulfide bridges are illustrated, and their positions noted above the diagram.



globulin class, and that generalizations derived from a study of κ , λ , and γ chains may not extend to comparisons of other immunoglobulin polypeptide chains.

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13. In the sequence comparisons, whenever the two human proteins differ in an amino acid assignment and the canine protein agrees with one of the assignments, the assumption has been made, for discussion purposes only, that the cause of disparity between the human sequences is of technical origin. For example, positions 386 to 8 were found to be Glu-Asn-Gly in the canine protein as well as in Ou. It is possible that the assignment Asp-Gly-Glu in the Gal protein represents a technical transposition. Similarly the deletion at position 373 and the insertion required between positions 488 to 489 may also be technical.
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Disturbance and the Dispersal of Fleshy Fruits

Abstract. *Fruits of Prunus serotina, Phytolacca americana, and Vitis vulpina were placed during separate trials in forest sites that varied in the degree to which the forest canopy was disturbed. Removal rates of fruits were consistently faster in the forest edge and light gap sites than in sites under closed canopy. Rapid removal of fruits from species that ripen fruit in summer and early fall is selectively advantageous to the plants because it minimizes the probability that fruits will be destroyed by invertebrates before dispersal. Disturbances probably play an important role in interactions between temperate fruits and birds and in community organization.*

Interactions between animals and plants are patchy in their occurrence. That is, individuals in certain locations in any plant population are more likely to be attacked by herbivores, to be visited by pollinators, or to have their seeds dispersed by animals than are plants in other locations in that population. Identification of the conditions under which these interactions are most likely to occur provides a basis for understanding the role played by interactions between animals and plants in generating or maintaining patchiness in communities.

The eastern deciduous forest of North America has been and is now a patchwork of stands that differ in age and structure (*1*). Small light gaps caused by treefalls and forest edges created by larger disturbances such as fire, tornadoes, or human activity interrupt areas of unbroken canopy. Although recent work has begun to unravel the role of disturbance in organizing the plant communities of temperate forests (*1*), little experimental work has been done on how this mosaic of patches affects interactions between animals and plants

(2). Many forest plants rely on animals for both pollination of flowers and dispersal of seeds. The purpose of our study was to evaluate the effect of this mosaic of patches on the probability of dispersal of fleshy fruits.

Many of the major avian frugivores either spend most of the year in areas with a well-developed shrub and vine layer (catbirds, brown thrashers) (*3*), or spend more time in such habitat types during late summer and early fall when most fruits are ripening than during spring and early summer (red-eyed vireos, some thrush species) (*4*). On the basis of these considerations, we hypothesized that fleshy fruits in light gaps and along forest edges have a greater probability of dispersal as compared to fruits under the closed forest canopy.

Experiments were conducted in Trelease Woods, a 22.4-ha preserve dominated by sugar maple (*Acer saccharum*) and located northeast of Urbana, Illinois. Sixty sites were chosen in three types of habitat. Under the closed canopy, 20 sites 50 m apart were distributed along two transects through the middle

of the forest. Twenty sites were also chosen along the forest edge, each separated by 100 m, with five sites on each of the four sides of the woods. Finally, 20 sites were chosen within light gaps in the forest (except for *Prunus* trial 1 in which only ten sites were used). All light gaps were 4 to 8 m in diameter and had a dense layer of vines.

An infructescence with a known number of fruits was taped to a branch of a shrub or tree about 2 m above the ground. The number of fruits per infructescence was the same for all sites on any one trial but varied between six and ten fruits between trials. The experiment was repeated five times between 31 July and 28 September 1977. This is the period over which most plants with fleshy fruits ripen in central Illinois. The experiment was performed twice with wild black cherry (*Prunus serotina*), twice with pokeweed (*Phytolacca americana*), and once with frost grape (*Vitis vulpina*).

These three species were chosen because of accessibility and morphological characteristics that made them easy to attach as multifruited units. Each trial lasted 7 days. The number of fruits remaining at each site, and the presence or absence of invertebrate damage was recorded daily. The ground beneath fruits was also checked daily for any fruits that may have fallen. The few fruits (< 1 percent) that did fall during the five trials were not counted as having been removed.

Fruits were removed at a significantly (*5*) faster rate from both the edge and light gap sites than from the sites under closed canopy (Fig. 1a). Only in the second *Prunus* trial did the rate of fruit removal from the edge sites not differ significantly from that under the closed canopy. Heavy thunderstorms over several days of this trial caused birds to move from the forest edge to the interior. During this trial, however, fruit removal within light gaps remained faster than under closed canopy as in all other trials. Whether edge or light gap sites had the faster removal rate varied between trials.

These differences in removal rates among habitat types have two components: (i) the rate at which sites were discovered (*6*), and (ii) the rate at which fruits are removed once sites were discovered. A significantly (*P* < .05) larger percentage of the sites under closed canopy as compared to both the edge and light gap sites remained undiscovered for a longer period of time (Fig. 1b). This suggested that sites resulting in rapid dis-