



# HOKKAIDO UNIVERSITY

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## ABSTRACTS

Abstract of Contributions on Biological Science from the Institute of Low Temperature Science Appeared or in Press in Publications other than *Low Temperature Science* (1980-1981)

SAKAI, A., D. M. PATON and P. WARDLE Freezing resistance of trees of the South Temperate Zone, especially subalpine species of Australasia. *Ecology*, 62 : 563-570 (1981)

*Abstract* The asymmetric distribution of land vs. sea between the Northern and Southern Hemispheres is reflected in the climates and the distribution of vegetation. The Southern hemisphere is characterized by having an oceanic climate with a relatively narrow annual range of temperature, cool summers and mild winters. The nearly total absence of deciduous hardwoods is characterized by the South Temperate Zone. And sub-cold coniferous forests and temperate deciduous broadleaved forests are characterized of the generally continental, winter-cold climates of North America and Eurasia. Little or no information on the winter hardiness of trees is available to compare the hardiness with trees native to the Northern Hemisphere.

Maximal resistance to winter freezing of trees of the South Temperate Zone, especially, Australia and New Zealand, including planted species native to Chile, was assessed. Most of the tree species which grow in lower altitudes were marginally hardy to  $-10^{\circ}\text{C}$ . Subalpine and alpine shrubby species such as *Podocarpus nivalis*, *P. lawrencei* and *Dacrydium bidwillii* were the hardiest conifers in New Zealand and Australia, resisting freezing to  $-20^{\circ}$  to  $-23^{\circ}\text{C}$ . The hardiness was comparable to that of conifers growing in warm temperate or temperate parts of Japan. In *Nothofagus*, the deciduous, subalpine *N. antarctica* of South America was the hardiest, resisting freezing at  $-22^{\circ}\text{C}$ . A New Zealand evergreen timberline species, *N. solandri* var. *clifortioides* was marginally hardy to  $-15^{\circ}\text{C}$ . Of the *Eucalyptus* species, *E. pauciflora* which forms the alpine tree limit on the mainland of Australia was the hardiest, resisting freezing to  $-15^{\circ}\text{C}$  in the leaves. Other high-altitude angiosperm species tested mostly survived freezing to only  $-10^{\circ}$  or  $-15^{\circ}\text{C}$ .

Very hardy tree species that withstand freezing below  $-30^{\circ}\text{C}$  seem not to have evolved in the Southern Hemisphere, because the mild, oceanic winter did not provide the stimulus to evolve very hardy species, and because hardy northern tree genera, with minor exceptions, have been unable to cross the barrier formed by the tropics.

SAKAI, A. and S. B. MALLA Winter hardiness of tree species at high altitudes in the East Himalaya, Nepal. *Ecology*, 62 : 1288-1298 (1981)

*Abstract* Nepal has a monsoon climate. The south sides of the mountain

ranges are almost always covered with drizzling mist and cloud in the summer. Although rainfall is sometimes nil in winter, atmospheric humidity is very high, especially in the morning and evening. The East Himalayan climate is characterized by a relatively small annual range of temperature, high rain fall in summer, rather high humidity, and moderate temperature in winter. Christchurch (43°42'S, 7 m altitude) in New Zealand has temperatures similar to those of Darjeeling in Sikkim (27°03'N, 2127 m altitude). Thus, as a whole, the East Himalaya experiences an oceanic climate although situated in inland areas. It may thus be assumed that very hardy conifers do not seem have evolved in the East Himalaya.

Little or no information on their winter hardiness of trees at high altitudes in the East Himalaya is available to classify these species as either oceanic or boreal in their response.

Maximal winter hardiness of trees in high-altitude forest zones, especially near timberlines in the East Himalaya, was assessed. A timberline coniferous species of the East Himalaya, *Abies spectabilis*, was marginally hardy to  $-20$  to  $-23^{\circ}\text{C}$ . *Larix potanii*, a high-altitude species which is the least hardy species of the genus *Larix*, also showed the same range of hardiness. The hardiness of these species is comparable to the least hardy Japanese Abietoideae that are native to the warm temperate forest zone. Subalpine rhododendrons such as *R. campanulatum*, *R. campylocarpum*, etc., were the hardiest of the Himalaya rhododendrons, resisting  $-20$  to  $-23^{\circ}\text{C}$  in their flower buds. These were less hardy than those occurring in the subalpine forests of Japan and North America. Thus in the Himalayas, rhododendrons and coniferous species, except *Pinus* and shrubby *Juniperus* seem not to have evolved species capable of resisting below  $-30^{\circ}\text{C}$ , unlike boreal and subalpine forests in central Japan, probably due to high humidity and moderate temperatures in the Himalayan winter and its floristic history.

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OOHATA, S., Y. HASEGAWA and A. SAKAI Studies on freezing resistance and distribution of the genus *Pinus* I. Seasonal changes in freezing resistance.\* Jap. J. Ecol., 31 : 79-89 (1981)

*Abstract* Seasonal variations of freezing resistance in 42 pine species which were planted at Kamigamo Experimental Station of Kyoto Univ. (35°01'N) were investigated with special reference to two thermal indices, mean warmth index (MWI) and mean temperature in the coldest month (MTCM) in native distributional regions. The timing of cold hardening in Kyoto differed significantly among pine species; The species from northern or colder regions became hardy earlier than those from southern or warmer regions. The winter hardiness was closely related to MTCM. The degree of hardiness was different from each other by the groups of pines from eastern and western regions of continents. The difference between them seems to be caused from difference of continentality of the climate. The winter hardiness

of pines appears to be more closely related to MTCM than MWI of their native habitats. Thus, it is postulated that very hardy pine species have evolved only in highly continental and winter cold climates. (\*In Japanese with English summary)

SATO, T. and A. SAKAI Cold tolerance of gametophytes and sporophytes of some cool temperate ferns native to Hokkaido. *Can. J. Bot.*, 59: 604-608 (1981)

*Abstract* To study the phenological adaptation of the life history of ferns to colder regions, the freezing resistance of gametophytes and sporophytes of 14 cool temperate region ferns native to Hokkaido was evaluated in Sapporo. Moreover, drought tolerance of gametophytes of some cool temperate ferns was elucidated. The mature sporophytes were hardy from  $-5$  to  $-20^{\circ}\text{C}$  in most species. However, gametophytes of most species were tolerant to below  $-40^{\circ}\text{C}$  and regrew into normal gametophytes in a month at room temperature. Most of the gametophytes survived well after dehydration to 2~10% water content. The gametophytic generation was more cold tolerant than the sporophytic one. Thus, the gametophytic generation, rather than the sporophytic, might be the generation that survives cold temperature in the field.

SATO, T. and A. SAKAI Observations on the spore-dispersal period of Pterophyta in Hokkaido.\* *Jap. J. Ecol.*, 31: 91-97 (1981)

*Abstract* Spore-dispersal period of Pterophyta is limited within one or two months in Hokkaido. Even in the same species, there is a tendency that the spore-dispersal period becomes shorter according to the forwarding in the northern part of Japan Archipelago. Many kind of summer-green ferns disperse spores from late July to September whereas other ferns including evergreen, semievergreen and winter-green ones from August to early november in Hokkaido. Among sixty Pterophyta species studied in Hokkaido, about forty species of them disperse spores during the limited period, from the end of August to early September. These characteristics of spore-dispersal might caused by the restriction of growing season in the cold climates. Moreover, many kinds of ferns which disperse spores at the end of August, usually grow into young gametophytes in early winter. This also suggests that the gametophytes should live in winter as thalli and wait for the alternation of generation into sporophytes until the coming spring and summer. These phenomena are regarded as one of the adaptive strategies in the early life history of gametophytic generation for overwintering of Pterophyta in the cold climates in Japan Archipelago. (\*In Japanese with English summary)

SATO, T. and A. SAKAI Freezing resistance of sporophytes of Pteridophyta in Hokkaido with special reference to their habitats.\* Jap. J. Ecol., 31 : 191-199 (1981)

*Abstract* To understand the adaptations to the low temperatures in the life history of Pteridophyta, the freezing resistance was evaluated with the overwintering sporophytes of fifty-five species native to Hokkaido. Most fern species increased their hardiness from September to December and reached the maximum in December. Evergreen ferns had their maximal leaf hardiness ranging from  $-12.5^{\circ}\text{C}$  to  $-40^{\circ}\text{C}$ , and the increase was much earlier and greater than that of the rhizome. Most summer-green ferns could not survive freezing in the leaf during October. The hardiness of the rhizome of the summer-green ferns ranged from  $-5^{\circ}\text{C}$  to  $-40^{\circ}\text{C}$  in Hokkaido. Almost all fern species growing on the floor of deciduous forest and in the humid and humus-rich dale had the hardiness ranging from  $-5^{\circ}\text{C}$  to  $-17.5^{\circ}\text{C}$ . It is known that the gametophytes of some ferns tolerated freezing even at  $-40^{\circ}\text{C}$ , while the sporophytes growing in the deciduous forest floor in Hokkaido survived freezing at the temperatures ranging from  $-5^{\circ}\text{C}$  to  $-17.5^{\circ}\text{C}$ . Thus, the present evidence foresees the possibilities of wintering by means of gametophytes on the cliffs and trunks, where it is extremely severe to winter in the state of sporophytes. (\*In Japanese with English summary)

YOSHIE, F. and A. SAKAI Freezing resistance of plants in relation to their life form and habitat.\* Jap. J. Ecol., 31 : 395-404 (1981).

*Abstract* Freezing resistance of 45 species growing in Hokkaido University Forest Station, Tomakomai was assessed to study the ecological adaptation to cold climates. Freezing resistance of most plant organs increases from September to late-December and decreases from February to late-May. The aerial organs were harder than the subterranean organs in most species examined. Phanerophytes were the hardest and geophytes were the least hardy in the freezing resistance of dormant buds. In rhizomes and roots, Hemicryptophytes growing in the road side were much harder than those in the forest floor, and geophytes were the least hardy. These hardiness trends seem to correspond to the temperature conditions surrounding the plant organs. On the other hand, little difference was found between hemicryptophytes in the road side and those in the forest floor, in the freezing resistance of dormant buds. These hardiness trends were still unchanged after hardening at  $-3^{\circ}\text{C}$  for 20 days from October to December. From these results, it seems that hardiness of the most plant organs is closely related to their life form and habitat, and determined by interaction to the hardiness and regeneration capacity of the other organs within individual. (\*In Japanese with English summary)

ISHIKAWA, M. and A. SAKAI Freezing avoidance mechanisms by supercooling in some *Rhododendron* flower buds with reference to water relations. *Plant & Cell Physiol.*, 22 : 953-967 (1981)

*Abstract* Excised florets of some hardy *Rhododendron* species did not withstand  $-5^{\circ}\text{C}$  due to intracellular freezing when they were artificially ice-inoculated. Florets in intact December buds, however, supercooled to about  $-30^{\circ}\text{C}$ . When flower buds of *R. japonicum* were slowly cooled with daily decrements of  $5^{\circ}\text{C}$  to temperatures ranging from 0 to  $-20^{\circ}\text{C}$ , exotherm temperatures of the florets drastically shifted to lower temperatures. This was accompanied by a decrease in water content of the florets and the peduncle and an increase in that of the scales where ice accumulated. The water in the florets and the peduncle is thought to migrate to the scales and other tissues during the stages of freezing of the scales and other tissues (this is considered to be a kind of extraorgan freezing); the dehydrated florets have a lower freezing point which enhances their supercooling ability and the dehydrated peduncle helps to maintain the supercooled state of the florets. This hypothesis would explain the dependence on the cooling rate of supercooling in *Rhododendron* flower buds. Water migration within flower buds was observed in other hardy *Rhododendron* species with some variation in ice formation site and the quantity of migrated water. The water translocation was more extensive in early winter and early spring when the floret water content was higher than in midwinter. The exotherm temperature of excised florets was inversely proportional to their water content. Dehydration of flower buds by wind at  $0^{\circ}\text{C}$  also enhanced their supercooling ability. Seasonal changes of the supercooling ability in *Rhododendron* flower buds seem to be attributed to the water migration from florets (and peduncle) to scales in response to freezing, which appears to be a unique adaptation of flower buds to freezing stress, and to the *in situ* desiccation of the buds by wind, etc. Mechanisms of freezing avoidance by supercooling in *Rhododendron* flower buds and the relationship of supercooling to freezing tolerance are discussed.

NIKI, T. and A. SAKAI Ultrastructural changes related to frost hardiness in the cortical parenchyma cells from mulberry twigs. *Plant & Cell Physiol.*, 22 : 171-183 (1981)

*Abstract* Ultrastructural changes in the cortical parenchyma cells of mulberry (*Morus bombyciz.* cv. 'Gorogi') twigs were related closely to the seasonal cycle of frost hardiness. Particularly, conspicuous changes in the plasmamembrane were demonstrated.

During stages that result in a rapid increase in hardiness, the sequence of cytological changes for the replacement of the plasmamembrane were observed as follows; marked invaginations of the plasmamembrane; pinching off into vesicles in the peripheral cytoplasm; engulfment of these vesicles

into the vacuole; generation of numerous microvesicles by the Golgi apparatus of these microvesicle in the peripheral cytoplasm and fusion of these microvesicle with the plasmamembrane. Similar cytological changes that are associated with increased hardness occurred during artificial hardening at 0°C for 5 to 20 days in both the autumn and spring. In midwinter cell, however, the plasmamembrane has a fairly smooth structure. Thus, the highly folded structure of the plasmamembrane is not always characteristic of extremely hardy cells.

Ultrastructural changes associated with hardness are discussed in terms of membrane renewal.

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SAGISAKA, S. The adenosine triphosphate levels of poplar during one year of the plant life. *Plant and Cell Physiol.*, 22: 1287-1292 (1981)

*Abstract* Adenosine triphosphate concentrations in poplar twigs (*Populus gelrica*) throughout one year were measured. Under natural conditions, the pool size of ATP in wintering plants was about 20 to 40 nanomoles per g dry weight xylem or living bark. At the time of growing and enlargement, the xylem contained more ATP (about 80 to 230 nanomoles per g dry weight) than that of the bark (about 160 nanomoles per g dry weight), indicating that the pool size of ATP and enzyme activities are controlled in such a way that they are able to proceed at a rate commensurate with demands of the cells. The values of adenylate energy charge were stabilized in the vicinity of 0.7 to 0.8 at about the time of onset of active growth in May, and also at the stage when the plant was almost ready for wintering in October or in November. Therefore, in winter, there was apparently no net increase in the total amount of adenylates, but the continued synthesis of ATP seemed to be occurring at the expense of ADP and AMP pools.

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SAGISAKA, S. and M. ASADA Coordinate and noncoordinate changes in enzyme activities in pentose phosphate cycle in poplar: A control of enzyme activities in differentiated xylem. *Plant and Cell Physiol.*, 22: 1459-1468 (1981)

*Abstract* Temporal controls in the expression of enzyme activities of pentose phosphate cycle in twigs of *Populus gelrica* were studied. Coordinate changes in the enzyme activities of the cycle (glucose-6-phosphate and 6-phosphogluconate dehydrogenases, transketolase, and transaldolase) occurred in the living bark and in the differentiating xylem cells. However, in early September in differentiated xylem cells, the former two dehydrogenase activities began to rise again, while the latter two transferase activities continued to decrease. The elevated levels of the two dehydrogenase activities in the differentiated xylem remained unchanged in the non-growing period of the life cycle of the plant. The occurrence of such differential

changes in enzyme activities at one particular time in the life cycle suggested that the pentose phosphate cycle in the differentiated xylem is interrupted in part at the step of transketolase reaction, which results in an increased supply of pentoses and NADPH. Moreover, these differential changes in the enzyme activities are correlated with changes in the fine structures of the cells.

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SOUZU H. Studies on the damage to *Escherichia coli* cell membrane caused by different rates of freeze-thawing. *Biochim. Biophys. Acta*, 603 : 13-26 (1980)

*Abstract* Freeze-thawing of *Escherichia coli* cells caused a release of cell membrane components such as protein, phospholipids and lipopolysaccharides. A greater amount of release and a lesser extent of cell survival were seen in slow freeze-thawing than in rapid freeze-thawing. Several dehydrogenases in the cells were also freed. The mode of release was also dependent on the rate of freeze-thawing.

The materials released by slow freeze-thawing were found to be mostly composed of outer membrane components, whereas the materials released by rapid freeze-thawing contained cytoplasmic as well as outer membrane components. The chemical composition of these fragments differed significantly from that of the original membranes. The relative content of cytoplasmic membrane-bound enzymes in these fragments also differed from that of the cytoplasmic membrane.

The fragmentation was assumed to have resulted mainly from the crystallization of external water. In slow freeze-thawing, it was considered that the phase separation of the membrane phospholipid bilayer increased the possibility of outer membrane fragmentation. Rapid freeze-thawing caused cytoplasmic membrane damage to the cells as well as to the outer membrane. In rapid freeze-thawing, the effect of phase separation appeared to be small because of rapid passage through the transition temperatures.

The presence of 10% glycerol completely inhibited the release of cellular materials and enzymes. Cell survival was maintained at a high level in the glycerol-treated samples whether freeze-thawed slowly or rapidly.

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FUJIKAWA, S. Freeze-fracture and etching studies on membrane damage on human erythrocytes caused by formation of intracellular ice : *Cryobiology*, 17 (4) : 351-362 (1980)

*Abstract* Ice crystals of 0.2 to 2.0  $\mu\text{m}$  in diameter were formed within cells as the result of rapid freezing of erythrocytes at the cooling rates around 8,000°C/min. Freeze-fracture and etching studies revealed the ultrastructural alteration of membranes by such intracellular ice. In the membrane regions which were in direct contact with intracellular ice, depressions resembling "worm-eaten spots" ranging from 400 to 3,000 Å in diameter were observed

both on etched PF and ES. The corresponding regions were covered by ice without etching. These results indicate that at the membrane regions in direct contact with intracellular ice, the path of fracturing was deviated from the inner plane of membrane due to the occurrence of molecular disorganization of bilayer membrane. The model for membrane structural alteration was proposed.

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FUJIKAWA, S. The effect of various cooling rates on the membrane ultrastructure of frozen human erythrocytes and its relation to the extent of haemolysis after thawing: *J. Cell Science*, 49: 369-382 (1981)

*Abstract* Human erythrocytes suspended in buffered isotonic saline were frozen to the temperature of liquid nitrogen at various cooling rates. As the cooling rates increased from 3 to 3,500°C/min, the extent of haemolysis gradually decreased, but further increase in cooling rates in excess of 8,000°C/min resulted in an abrupt increase of lysis.

Membrane-associated vesicles devoid of intramembrane particles were formed in the erythrocyte membranes frozen at cooling rates slower than 1,500°C/min. The frequency and size of these vesicles were highly cooling rate dependent. Another membrane ultrastructural change associated closely with the formation of intracellular ice crystals appeared at cooling rate faster than 8,000°C/min. The erythrocyte membrane frozen at a cooling rate of 3,500°C/min exhibited ultrastructural integrity by avoiding the membrane changes caused by either slow or fast freezing. It is suggested from the close relation between membrane ultrastructure and extent of haemolysis that the ultrastructural integrity of membrane in the frozen state is important for avoiding haemolysis after thawing, and that the membrane ultrastructural changes caused by both slow and fast freezing were responsible for the lysis after thawing.

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SAKAGAMI, Sh. F. Bionomics of the Halictine Bees in Northern Japan. I. *Halictus (Halictus) tsingtouensis* (Hymenoptera, Halictidae), with Notes on the Number of Origins of Eusociality. *Kontyû*, Tokyo, 48(4): 526-536 (1980)

*Abstract* In Sapporo, northern Japan, the halictine bee *Halictus (Halictus) tsingtouensis* STRAND is univoltine, solitary and polytrophic with brood rearing from mid June to mid July. The brood cells are directly connected to the main burrow as in the nests of many consubgeneric species. In the halictine bees, the probability of independent origins of eusociality in related phyletic groups should increase when more solitary species are included in these groups. In connection with this assumption, necessity of precise life cycle records of more halictine species, not only social but also solitary ones, is stressed.

SIMÕES, D., L. R. BEGO, R. ZUCCHI and Sh. F. SAKAGAMI *Melaloncha sinistra* Borgmeier, an endoparasitic phorid fly attacking *Nannotrigona (Scaptotrigona) postica* Latreille (Hymenoptera, Meliponinae). Rev. Bras. Ent., 24: 137-142 (1980).

*Abstract* Parasitism by the phorid fly *Melaloncha sinistra* on the stingless bee *Nannotrigona (Scaptotrigona) postica* is reported. The immatures of the fly are endoparasitic on the foraging workers. In heavy cases, infestation attains 34% of total foragers, resulting in a drastic decrease of colony activities. Besides bionomic notes, the puparium is compared with that of *M. ronmai* parasitic on the honeybee.

SAKAGAMI, Sh. F. and M. ITO Specific and subgeneric variations in tibial corbication of male bumblebees (Hymenoptera: Apidae), an apparently functionless character. Ent. Scand. Suppl. 15: 365-376 (1981)

*Abstract* The male hind tibiae of bumblebees exhibit diverse conditions ranging from the fairly corbicated state resembling that in females to the opposite extreme. Comparison of 105 species covering most subgenera shows the prevalence of more corbicated states in Section *Anodontobombus* (particularly in *Bombus* S. Str.) than in Section *Odontobombus*, although within the latter *Megabombus* and *Diversobombus* exhibit the most corbicated state, and a segregation of the states in *Fervidobombus*. The hind tibiae of both sexes of most apid genera are illustrated. Based upon four assumptions a tentative hypothesis explaining the evolution of male corbication is presented. In reference to the importance of subgeneric differences in comparative studies of bumblebees, comments are given on the study by Stiles (1979) on the color pattern and pubescence characters in males.

TAKADA, H. and M. J. TODA Notes on Arctic Canadian Diastatidae and Drosophilidae (Diptera), with the description of a new species. J. Fac. General Edu. Sapporo Univ., 18(A): 1-8 (1981)

*Abstract* The list of three species of Diastatidae and thirteen species of Drosophilidae collected in the Arctic Canada is reported, together with collection records and the description of a new species, *Amiota quadrata* sp. nov.

HOSHIKAWA, K. Climatic "host race" formation in *Henosepilachna vigintioctomaculata* complex, a sibling group of phytophagous ladybirds (Coccinellidae: Coleoptera). XVI International Congress of Entomology, Abstracts p. 11, (1980)

*Abstract* Several closely allied species of Epilachninae occur in Japan, varying in morphology and food habits, being divided into two main groups.

One consists of a single species, *H. 28-maculata*, distributed in Eastern Asia. The other is endemic to Japan and considered to be diversified there, involving several races or species. Their food preferences vary geographically, including some clines. An analysis on the mechanism of cline formation proved that relative food shortage, in relation to bionomic discordance between insects and plants, is a cause of niche expansion. This discordance seems to be brought by the discrepancy of climatic adaptation between insects and plants. Moving zone of the food shortage divides a race into two host races, as a wedge on adaptation to the host plant. In reference to Quaternary climatic change, phylogenetic relations of these host races could be interpreted by geographic variations in food preference.

CHINO, H., H. KATASE, R. G. H. DOWNER and K. TAKAHASHI Diacylglycerol-carrying-lipoprotein of hemolymph of the American cockroach: Purification, characterization and function. *J. Lipid Research*, 22 : 7-15 (1981)

*Abstract* A diacylglycerol-carrying lipoprotein (DGLP) was isolated and purified from the hemolymph of adult male and female American cockroaches, *Periplaneta americana*. The purification procedure involved dialysis against distilled water, precipitation at low ionic concentration, and separation by column chromatography on DEAE-cellulose. The final preparation was homogeneous as judged by polyacrylamide gel electrophoresis and electron microscopy. The lipoprotein comprised over 50% of the total hemolymph protein. The DGLP molecule was almost globular in shape with a diameter of approximately 160 Å. The molecular weight was approximately 600,000. Apoprotein of DGLP consisted of two non-identical subunits, heavy chain (mol wt 250,000) and light chain (85,000). The total lipid content of DGLP amounted to about 50%. The lipids comprised diacylglycerol (15% of total lipid), hydrocarbon (28%), cholesterol (5%), and phospholipid (43%). *n*-Pentacosane, 3-methylpentacosane, and 6, 9-heptacosadiene were identified as major hydrocarbons. Mannose (0.9%) and glucosamine (0.3%) were associated with apoprotein of DGLP. The capacity of the DGLP to accept diacylglycerol from both fat body and midgut was demonstrated *in vitro*; thus, it was suggested that the same carrier molecule serves to transport this lipid from storage site and absorption site. The possible multiple function of cockroach DGLP in transporting such lipids as diacylglycerol, cholesterol, and hydrocarbon from the site of storage, absorption, or synthesis is discussed.

CHINO, H. and K. KITAZAWA Diacylglycerol-carrying lipoprotein of hemolymph of the locust and some insects. *J. Lipid Research*, 22 : 1042-1052 (1981)

*Abstract* The diacylglycerol-carrying lipoprotein (DGLP) was purified from

placed at 2°C for several weeks, the hemolymph trehalose content increased included a specific precipitation at low ionic concentration and DEAE-cellulose column chromatography. The final preparation was highly homogeneous as judged by gel electrophoresis, electron microscopy, and immunodiffusion. The locust DGLP molecule was almost spherical in shape with a diameter of about 130 Å. The molecular weight was approximately 580,000. The total lipid content amounted to about 40%. The lipid comprised diacylglycerol (33% of total lipid), hydrocarbon (21%), cholesterol (8%), and phospholipids (36%). The hydrocarbon fraction contained a number of *n*-alkanes and methylalkanes ranging from C<sub>25</sub> to C<sub>38</sub> in chain length. Mannose (3%) and glucosamine (0.5%) were associated with the apoprotein of DGLP. Apoprotein of locust DGLP consisted of two non-identical subunits, heavy chain (250,000) and light chain (85,000); carbohydrate was associated only with the heavy chain. Tests of physiological function of DGLPs from locust, cockroach, and silkworm suggest that the insect DGLP serves multiple function as a true carrier molecule in transporting diacylglycerol, cholesterol, and hydrocarbon from the sites of storage, absorption, and synthesis to sites where these lipids are utilized as metabolic fuel, precursors for triacylglycerol and phospholipids synthesis, or structural components of cell membrane and cuticle. In addition, the insect DGLPs displayed no species specificity in terms of function, whereas they were immunologically distinguishable.

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CHINZEL, Y., CHINO, H. and G. R. WYATT Purification and properties of vitellogenin and vitellin from *Locusta migratoria*. *Insect Biochemistry*, 11: 1-7 (1981)

*Abstract* The vitellogenin (VG) and vitellin (VN) of *Locusta migratoria* have been purified from ovariectomized female hemolymph and from oocytes, respectively, by a new procedure. The products are free of diacylglycerol-carrying lipoprotein, which was present as a contaminant in previous preparations. The isoelectric point of VG and VN is pH 6.3. The amino acid compositions of VG and VN do not differ significantly. The lipid moiety (8.2% for VG and 6.7% for VN) includes diacylglycerol, a trace of triacylglycerol, cholesterol, and phospholipids. The content of diacylglycerol is much lower in VN than in VG, which suggests the release of lipid within the oocytes.

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HAYAKAWA, Y. and H. CHINO Temperature-dependent interconversion between glycogen and trehalose in diapausing pupae of the *Philosamia* silkworm. *Insect Biochemistry*, 11: 43-47 (1981)

*Abstract* A temperature-dependent interconversion between fat body glycogen and hemolymph trehalose was demonstrated in diapausing pupae of the silkworm, *Philosamia cynthia*. When pupae at early diapause stage were

hemolymph of the locust, *Locusta migratoria*, by a rapid method which to about 35~50 mg/ml of hemolymph, whereas the trehalose content of insects maintained at 20°C remained at 5~10 mg/ml. Concomitant with this change of hemolymph trehalose level, the glycogen content of fat body dropped from 29~41 mg to 6.6~8.6 mg/g wet weight. This interconversion could be demonstrated repeatedly if the diapausing pupae were successively exposed to high and low temperatures, although the total amount of carbohydrates decreased slightly during repeated interconversions. Nondiapausing pupae of the silkworm, *Philosamia ricini*, did not accumulate trehalose appreciably even when exposed to 2°C for a long period.

CHINO, H., R. G. H. DOWNER, G. R. WYATT and L. I. GILBERT Lipophorins, a major class of lipoproteins of insect hemolymph. *Insect Biochemistry*, 11 : 491 (1981)

*Abstract* Two classes of lipoprotein with different physicochemical properties and biological functions have been isolated and characterized from the hemolymph of several insects. One of these is the female specific lipoprotein, vetellogenin. The second has been described as diglyceride-carrying lipoprotein, high density lipoprotein, and lipoprotein I, although the first of these terms has been most widely accepted. Since this lipoprotein is an abundant constituent of insect hemolymph and is the subject of much current research directed toward the analysis of its structure, functions and biosynthesis, a simple and appropriate name for it is desirable. Recent observations in the American cockroach, silkworm and locust indicate that this lipoprotein serves as a true carrier molecule in transporting hydrocarbon and cholesterol in addition to diacylglycerol. The demonstrated multiplicity of functions renders the term of diacylglycerol-carrying lipoprotein inaccurate. Therefore, we propose "Lipophorin" (in Greek, *lipos* lipid; *phoros* bearing) as an appropriate generic term for this unique lipoprotein of insect hemolymph.

CHINO, H. Lipid transport by hemolymph lipoprotein: A possible multiple function of diacylglycerol-carrying lipoprotein.

A chapter contributed to the Book, *Metabolic Regulation in Insects*, (R. G. H. Downer, ed.), Plenum Press, N. Y. (1981)

KATAGIRI, C., J. S. OWEN, P. J. QUINN and D. CHAPMAN Hydrogenation of plasma lipoproteins by a water-soluble catalyst; its use as a structural probe. *Eur. J. Biochem.*, 118 : 335-338 (1981)

*Abstract* Hydrogenation of double bonds of fatty acyl chains in the lipids of individual, intact plasma lipoproteins has been accomplished using a water-soluble, homogeneous catalyst. Up to 40% of the double bonds can be

hydrogenated. The pattern of hydrogenation is similar for each of the lipoprotein fractions; the phospholipids are most extensively hydrogenated but small amounts of cholesteryl ester and triacylglycerol are also hydrogenated. This result is consistent with a surface location of phospholipids and with the majority of the apolar lipids being in a hydrophobic inner core since the catalyst was shown not to be specific for an individual lipid class. It also suggests that some cholesteryl ester and triacylglycerol may be near the lipoprotein surface, accessible to the catalyst. If this is the case, it is speculated that the known exchange of these neutral lipids between the lipoprotein classes and their degradation by lipolytic enzymes may thus be facilitated.

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HOSHI, M., T. NUMAKUNAI and H. SAWADA Evidence for participation of sperm proteinases in fertilization of the solitary ascidian, *Halocynthia roretzi*: Effects of protease inhibitors. *Developmental Biology*, 86 : 117-121 (1981)

*Abstract* Effects of 15 proteinase inhibitors and an inhibitor against aminopeptidases on fertilization of the solitary ascidian, *Halocynthia roretzi* were studied in search of lysins. Fertilization of intact eggs was blocked by three trypsin inhibitors, leupeptin, antipain, and soybean trypsin inhibitor, and by two chymotrypsin inhibitors, chymostatin and potato proteinase inhibitor I. On the other hand, the fertilization of naked eggs was not blocked at all by leupeptin and was only partially blocked by chymostatin at the concentrations sufficient for blocking that of intact eggs. This indicates that spermatozoa utilize trypsin-like and chymotrypsin-like proteinases probably as lysins for penetrating through the chorion. The chymotrypsin-like activity appears to be also required for some step besides sperm penetration through the egg investments.

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DALE, B., M. DAN-SOHKAWA, A. DE SANTIS and M. HOSHI Fertilization of the starfish *Astropecten aurantiacus*. *Exp. Cell Res.*, 132 : 505-510 (1981)

*Abstract* In the starfish *Astropecten aurantiacus* the acrosome reaction occurs when the spermatozoon contacts the outer surface of the jelly layer. A long thin acrosomal filament is extruded from the anterior region of the spermatozoon and establishes contact with the oocyte surface. This latter interaction initiates the movement of the spermatozoon to the oocyte surface, formation of the fertilization cone and the cortical reaction. The first detectable electrical change across the oocyte plasma membrane during interaction with the spermatozoon is the fertilization potential (FP) which occurs simultaneously with the cortical reaction. The FP is probably the electrical result of the modification of the oocyte plasma membrane during cortical exocytosis. There are no primary step-like depolarizations during fertilization

of starfish oocytes, which contrasts with the situation in sea urchin eggs. We suggest that the difference in electrical response to fertilization of starfish oocytes and sea urchin eggs may be attributed to the location of the acrosome reaction in these animals and not to their different meiotic states.

IKADAI, H. and M. HOSHI Biochemical studies on the acrosome reaction of the starfish, *Asterias amurensis* I. Factors participating in the acrosome reaction. *Develop., Growth and Differ.*, 23 (2): 73-80 (1981)

*Abstract* In contrast with the case in sea urchin sperm, in starfish the acrosome reaction is not spontaneously induced by simply increasing the extracellular  $\text{Ca}^{2+}$  concentration of pH. At higher pHs, starfish sperm undergo morphological changes accompanied by exocytosis of the acrosomal vacuole, but they do not form acrosomal filaments. Nomarski-microscopic observation confirmed that spermatozoa undergo the acrosome reaction within the jelly coat. Acrosome reaction-inducing substance, a glycoprotein from the egg jelly, required a diffusible cofactor(s) present in the egg jelly for full activity. Several lines of evidence showed that this diffusible factor(s) is not merely  $\text{Ca}^{2+}$ .

IKADAI, H. and M. HOSHI Biochemical studies on the acrosome reaction of the starfish, *Asterias amurensis* II. Purification and characterization of acrosome reaction-inducing substance. *Develop., Growth and Differ.*, 23 (2): 81-88 (1981)

*Abstract* The acrosome reaction-inducing substance (ARIS) was purified from egg jelly of the starfish, *Asterias amurensis*. The purification procedure included elimination of neutral glycoproteins from the ARIS fraction by isoelectric point precipitation and subsequent gel filtrations on Sephadex G-50 and Bio-Gel A-50 m columns. The final preparation of ARIS was homogeneous as judged by cellulose acetate electrophoresis of ARIS and by ion-exchange chromatography on DEAE-Sephadex A-25 of S-carboxymethylated ARIS. ARIS is a very large, sulfated glycoprotein containing fucose, galactose, galactosamine and glucosamine as sugar components. It requires a diffusible cofactor (Co-ARIS) for full biological activity. A Pronase digest of ARIS retained its capacity to induce the acrosome reaction when Co-ARIS was added to the bioassay system. The physiological significance of the carbohydrate moiety of ARIS is discussed.