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第 13 回談話会(蒲郡)会記

開 催 地 愛知県額田郡幸田町遠望峰 三河ハイツ

期 日 昭和 52 年 8 月 20 日(土)・21 日(日)

日 程 8 月 20 日、午前 10 時 20 分頃高岡・大木・岩井川 3 名会場に到着、諸準備に取りかかる。参加者の大部分は 12 時 30 分蒲郡駅に集合、三河ハイツのマイクロバスで会場に到着、数名が個別に参集した。参加総数 22 名、講演題数 9。今回は特に米国モンタナ州立大学教授ピツシャー夫妻も参加された。午後 1 時 45 分から 5 時まで一般講演 4 題とピツシャー教授の特別講演を行う。6 時 30 分より午後 11 時過ぎまで、会食懇親会を行う。大鼓など叩きながら各種の歌を合唱し、盛大な会となった。

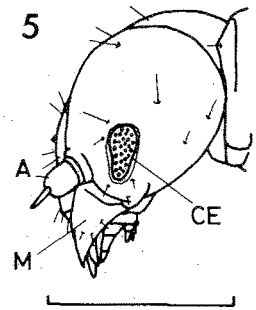
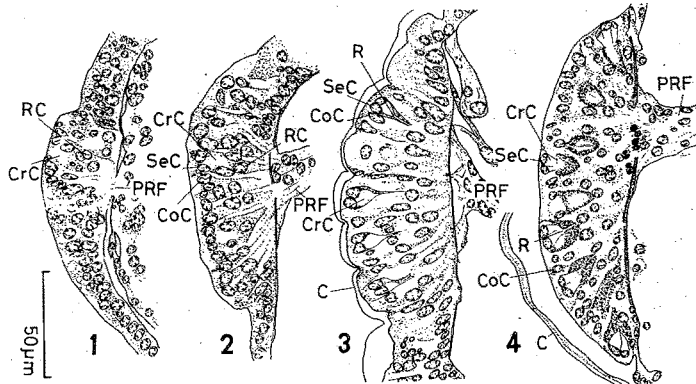
8 月 21 日、午前 9 時より総会に入り、来年度開催予定地・世話人を決定し、講演要旨集の処理方法等を決定(前回分については前例通り、今回は印刷その他を大木が担当)。午前 10 時より 2 日目の一般講演に移る。途中 11 時 30 分ピツシャー夫妻と安藤氏が関西方面へ出発のため、講演を一時中断したが、12 時 30 分、予定した講演をすべて終り、昼食後解散した。

ブライアシリアゲ *Panorpa pryeri*
MacLachlan (長翅目)の幼虫眼の
胚子発生

安藤 裕(筑波大学 菅平高原実験センター)
鈴木 信夫(筑波大学 生物科学系)

完全変態昆虫類中、最も早く分化したと考えられる長翅目の発生については、今日までほとんど知見がない。そこで、今回は長翅目で特に興味深いこの虫の幼虫眼が真正の複眼であるか否かを確認するために、胚子発生をブライアシリアゲ *Panorpa pryeri* MacLachlan を材料にして観察してみた。

ブライアシリアゲの卵期は、約7日(21℃)であるが、幼虫眼は産卵後4日目頃から、頭部両側の外胚葉の肥厚(将来の眼域)と共に分化がはじまる。図1は、やや発生が進んだ産卵後5日の眼域で、透明の部分が晶子体(CrC)、周辺の細胞が、分化中の網膜細胞(RC)、色素細胞などである。また、この幼虫眼原基と第1大脳の視葉を結ぶ後網膜神経繊維(PRF)が見られる。産卵後6日(図2)になると晶子体・網膜細胞はさらに発達し、角膜原細胞(CoC)やゼンパー氏細胞(SeC)も分化し、それぞれの個眼を明瞭に識別できるようになる。孵化直前(図3)の幼虫では個眼各部はさらに発達し、円錐形の晶子体の下に桿状体(R)が、また幼虫眼の表層には角膜(C)が形成される。孵化直後の幼虫眼は、色素細胞の色素も濃くなり、約30の個眼が完成し、機能的な複眼となる。図5は1令幼虫の頭部を示したものである。



A: antenna, CE: compound eye, M: mandible.

以上、ブライアシリアゲの幼虫眼の発生過程の概略を述べたが、これは明らかに、他の昆虫で知られている複眼形成過程と同様で、側単眼の形成過程とは、大そう異質なものであることを、広翅目のヘビトンボ・ネグロセンブリ、脈翅目のキバネツノトンボ、鱗翅目の最も原始的なグループに属するヒロコバネの幼虫眼と比較して説明した。

ヨーロッパのシリアゲムシの1種 *P. communis* L. の幼虫眼が複眼であることは、既に BIERBRODT(1943) が報告しているが、今回の胚子発生過程からも、シリアゲムシの幼虫眼が複眼であることが確かめられた。一般に幼虫期に複眼を持つ昆虫は不完全変態類に限られており、完全変態類の幼虫は、複眼のかわりに、側単眼を持つことは周知のことである。ところが完全変態類中でシリアゲムシ類の幼虫は例外的に複眼を持つ点が極めて特異的である。シリアゲムシ類の幼虫が土中にトンネルを掘り生活するにもかかわらず、不完全変態類の幼虫のように、発達した複眼を持つという事実は、完全変態類中、最も古いグループの一つと考えられるこの昆虫の系統を考察する上で重要なことであろう。

第13回講演要旨

ガロアムシの視覚器官

長島 孝行(東京農業大学昆虫学研究室)

後閑 暢夫(//)

日本で最も一般的な *G. nipponensis* の眼の微細構造を観察した。眼は38個の個眼が楕円状にラフに集合して形成されている。眼域の表面には体壁と同様の多数の小孔があり、そこからロウ物質と思われる「カラ草」状の構造物が生じている。角膜は両凸レンズ状を呈しているが、一般体壁同様 pore canal が存在し、角膜レンズとしての分化の程度は低いものと思われる。

視細胞は個眼により数は一定ではないが、多くの場合12個よりなる。これは上下2層に分れて配列されており、それぞれにラドメアーを有し、集合型のラドームを形成している。ラドメアーの microvilli は配列が不規則で基部においては、非常に乱れて空胞状を呈している。色素細胞は primary, secondary の明瞭な識別ができない。

第13回談話会(蒲郡) 参加者(ABC順)

安 藤 裕	筑波大学菅平高原実験センター
後 閑 暢 夫	東京農業大学昆虫学研究室
芳 賀 和 夫	筑波大学生物科学系
伊 藤 富 夫	静岡大学教育学部生物学教室
伊 藤 義 昭	愛知医科大学生物学教室
岩井川 幸 生	名古屋大学教養部生物学教室
近 藤 昭 夫	東邦大学理学部生物学教室
松 崎 守 夫	福島大学教育学部生物学教室
宮 慶一郎	岩手大学農学部応用昆虫学教室
宮 川 幸 三	学習院中等部
長 島 孝 行	東京農業大学昆虫学研究室
大 木 健 市	名古屋大学教養部生物学教室
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関 口 晃 一	筑波大学生物科学系
高 岡 実	愛知学院大学生物学教室
田 中 正 弘	岐阜市 加納高等学校
Prof. S.N. Visscher	米国モンタナ州立大
Prof. P. Visscher	//
矢 島 英 雄	茨城大学理学部生物学教室
山 崎 柄 根	埼玉大学教育学部生物学研究室
安 富 真 澄	愛知医科大学生物学教室

第13回講演要旨

ヘビトンボの発生、特に胚子の発生にともなう外部形態変化について

宮川 幸三(学習院)

広翅目(Megaloptera)の発生に関する報告はSTRINDBERG(1916)とDUBOIS(1938)のセンプリ(*Sialis lutaria* L.、センプリ科)に関するものがあるのみで、より原始的なヘビトンボ科については全く研究されていない。

演者は1976年8月27日、東京都下奥多摩町でヘビトンボ*Protohermes grandis* THUNBERG 1♂、2♀♀を夜間採集し、当夜1♀が数百個の卵を産んだので、それをもとにして、本種の発生の観察を行いつつある。

卵殻は薄く、初めは透明であるが後に着色するとともに不透明になる。この場合も流動パラフィンに浸けると透明化するので、生卵の観察はこの方法によった。固定はFAAにより、切片は通常のパラフィン法、染色はMAYERのヘマラウムとエオシンを用いた。

胚期は室温(24°C±4°C)で13日であった。以下胚子の外部形態変化を略記する。

- 第1日 受精、卵割
- 第2日 胞胚~胚帯形成
- 第3日 胚帯の伸長、体節化開始、胚膜形成完了、付属肢出現
- 第4日 卵殻着色(前極腹側にV字状白線)
- 第5日 胚子反転開始

第6日 頭部の形態形成開始

第7日 眼の形成開始

第8~9日 眼の着色強化、背部閉鎖、付属肢の関節形成

第10日 付属肢ほぼ完成

第11日 胚脱皮

第13日 孵化、卵殻のV字状白線が破れて胚子(幼虫)脱出

ヘビトンボ卵は腹側にやや突な円筒形(長さ1.25mm、直径0.53mm)で、前極に多数の卵門をもつ大きな突出部がある。この形はセンプリのそれに酷似する。発生の様式や経過もセンプリの場合と同様で、それぞれ全胚期を同一基準にとって比較すると、胞胚期がヘビトンボでやや長いほかは両者ほとんど差がない。特に初期胚帯の位置と形状、羊膜褶の発生、胚膜の形成過程は両者でよく似る。また、胚膜の完成が遅く胚帯に体節化が開始された後である点は脈翅目のクサカゲロウ

Chrysopa peria L.(BOCK、1939)とも似る。胚子反転が腹部後端に始まり前方に及ぶ様式は、脈翅目(BOCK、1939)、トビケラ目(PATTEN、1884; MIYAKAWA 1973)、ノミ目(KESSEL、1939)の場合と類似する。

第13回講演要旨

ヒシモンユスリカ (*Chironomus samoensis* Ednards) 発生初期胚後端への紫外線照射による双頭胚 (Double Cephalon) 形成

矢島 英雄 (茨城大学理学部生物学教室)

著者は先にユスリカ発生初期胚の卵前部、卵後部に対するその側方よりのUV照射より、それぞれ双腹胚 (Double Abdomen)、双頭胚 (Double Cephalon) を得たが、それらの出現頻度はそれぞれ最高22.2%、5.2%と低かった ('64)。

その後 Kalthoff & Sander ('68) はユスリカ的一种 *Smittia spec.* 卵で著者と同様の方法で双腹胚を得ることに成功し、さらに Kalthoff ('69~'76) はその後の一連の研究より、i) UV処理後の低温飼育 (8°C) が双腹胚出現頻度を高めること、逆に云えば高温飼育 (24°C) ではUV効果が無くなり正常胚になってしまうので、この現象を温度回復 "Thermoreversion" と呼んだ。ii) 双腹胚形成は極細胞形成以前から、Pre-blastoderm期 (Syncytial blastoderm' st. ?) にかけて得られること。iii) その効果標的は前半1/4以内の卵表層の核以外の部分に存在すること、iv) 奇形形成の作用スペクトルは280nmにpeakがあり、260nmに肩がみられること、およびRNase処理が効果あることから、ターゲットはRNPであると考えに至った (Kandler-Sniger & Kalthoff, '76)。v) しかしながらこれら一連の研

究の中でも繰返し述べているように、双頭胚 (Double Cephalon) の形成にはついに成功せず、彼の画いたユスリカ環節パターン形成の説明図式は不完全なものに終わっている (Kalthoff, '76)。

今回著者は双頭胚を従前より高頻度で得ることに成功したので、ここに報告するが、それは、i) UVによる処理時期が極めて限られること、核移動後10~30分の範囲内にある。ii) 照射方法は卵後方よりとした。iii) UV処理後 Kalthoffにより培養温度を低くすると双頭胚出現頻度が高まる。すなわちこの場合にも双腹胚形成の場合と同じく、"Thermoreversion" の現象が認められる。iv) 高率で光回復する。この点も共通である。以上より双頭胚・双腹胚形成に関するUVターゲットは共通であることが考えられる。しかしながら、その細かい点では反応行動に差がみられ、この差が頭部形質、腹部形質決定につながるのであろう。

第13回講演要旨

シヨウジョウバエ極顆粒分画の分離

I. 分画の性状

岡田 益吉(筑波大学生物科学系)

極細胞形成および生殖細胞決定をおこなう物質はどこに存在するかを明らかにする目的で、卵のhomogenateを分画し、上記の能力のある分画をbioassayを使って選択した。その結果、27,000g 1時間遠心で沈澱する分画が最も強い極細胞形成能があることを発見した。この分画の持つ性状を研究中であるが、その一部を報告する。なお、この研究は上田竜(筑波大、DC3年)、仁木雄三(筑波大、DC2年)が私の指導の下におこなったものである。

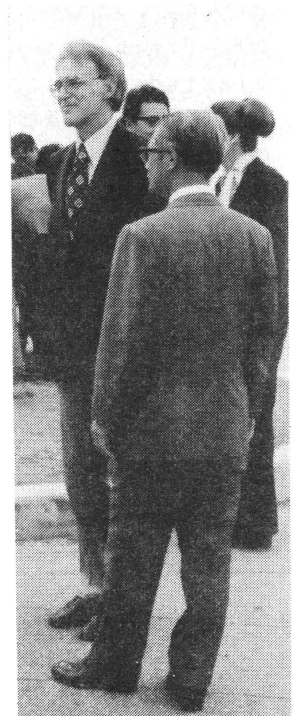


第13回談話会

昭和52年8月21日

愛知県幸田町

三河ハイッ



第13回講演要旨

カプトガニの体節増加胚の実験的形成

伊藤 富夫(静岡大学教育学部生物学教室)

関口 晃一(筑波大学生物科学系)

Cytochalasin Bや α -lipoic acidなどによる処理で、カプトガニ胚に体節数の減少がみられることを、以前、当会において報告した(1974~1975)。今回、逆に体節数の多いカプトガニ胚を実験的に形成できたので報告する。

1) NaHCO_3 による体節増加胚:

NaHCO_3 を含んだ海水による処理で、体節増加胚が形成された。この体節増加胚は、頭胸部最後の第6体節のあとに、さらに付属肢を伴った1つの体節をもっていた。増加した体節は、第6体節の後側に新たにできたもののみで、他の体節部がふえたものではない。増加した体節は痕跡程度のものから、完全といえるものまでであったが、完全に近い増加体節の付属肢は頭胸部第6肢と同様な形態をしていた。

濃度: 0.1Mが、もっとも有効。0.01M以下では正常に発生し、0.2M以上での処理では発生が進まなかった(処理時間: 24時間 発生段階: 体節出現期)

処理時間: 著しい形態形成運動がおわり、体節構造が出現してくる時期(Stage 12)に有効であり、他のStageでの処理では生じなかった。

出現頻度: 最適の条件で、約20%の率で生じた。

NaHCO_3 の各構成イオンのうち、有効なものは CO_3 イオンであった。 CO_3 イオンは、エネルギー代謝系の抑制作用や Ca^{++} イオンとの結合作用などの働きをするといわれるが、エネルギー代謝のInhibitor類での処理では、体節増加胚は生じなかった。逆に、 Ca^{++} イオンは NaHCO_3 の体節増加胚を生じさせる作用を抑え、また、 Ca^{++} free海水に Ca^{++} イオンのキレート剤EGTAを加えたものによる処理で、体節増加胚が形成

された。これらの点から、 NaHCO_3 の体節増加胚の形成作用は Ca^{++} イオンをカプトガニ胚から奪うことに基づくと考えられる。

2) Actinomycin Dによる体節増加胚: 体節の増加は、Actinomycin Dによる処理でも生じた。この場合の体節の増加も、頭胸部の最後端に付加して、腹部との間にわりこんだ形でおきていた。ただしほぼ完全な増加体節の付属肢は、第6肢と同様な形態をしたもの他、エラ状の構造物をつけた肢もあった。また、増加した体節数は2つのものもあった。

濃度: 1~10 $\mu\text{g}/\text{ml}$ の処理で有効であった。(時間: 24時間、発生段階: 体節出現期)。

処理時間: 24時間が最も有効であった。(濃度: 5 $\mu\text{g}/\text{ml}$ 、発生段階: 体節出現期)。

発生段階: 著しい形態形成運動による胚域の伸長がおわり、体節構造が出現してくる時期(Stage 12)に有効であった。

Actinomycin Dによる体節増加胚形成の原因は、考察の段階でない。また、 NaHCO_3 による増加胚とActinomycin Dによる体節増加胚の原因の関連性も不明である。

なお、PuromycinやCycloheximideなどタンパク合成阻害剤、HydroxyureaなどDNA合成阻害剤では、体節増加胚は生じなかった。

第13回講演要旨

カイコ卵の初期胚形成の電顕的観察

I. 成熟および受精

宮 慶一郎(岩手大学・農学部)

産下直後のカイコ卵の構造および精子の侵入に伴うその変化を電子顕微鏡で観察した。

卵殻は既に知られているように外、中、内の3層からなるが、卵門部では外層の他に海綿状構造の内層をも欠除している。卵殻の直下には電子密度の高い層状の卵黄膜があり、これと卵細胞膜との間の広い“膜間層”には多数の不規則な高電子密度顆粒が散在し、これは卵門部において特に著しい。

卵細胞膜に包まれて周辺原形質層が存在するが、光顕的にも一様の構造ではなく、比較的薄い層の所々に大型の好塩基性顆粒が散在している。電顕的には細胞質礎質の薄層中にはミトコンドリア、リボゾーム、少数のグリコゲン顆粒、脂質小滴等が含まれ、好塩基性顆粒に相当する部域には、ミトコンドリア、

リボゾーム、粗面小胞体の集積が多く、その他小管状構造を含む小体、多胞体様構造、厚膜顆粒等が含まれている。これらの小器官、特に小胞体、リボゾーム、ミトコンドリアの形態や分布に卵域による差が認められる。

精子の侵入に伴う卵表層の構造の変化は最初卵黄膜と膜間層顆粒に現われ、前者は著しく薄くなり、後者は卵前極部から消失し始める。続いて周辺原形質中の好塩基性顆粒が崩壊し始めるが、これは電顕的には小胞体集積の展開として認められる。

以上の結果から、精子の侵入と卵黄膜、膜間層との関連性、卵域による構造の差異と胚盤葉期における細胞分化の問題追求の可能性が示唆された。

オオカマキリ精子の微細構造

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カマキリ類では生殖細胞がどのようにしてつくられるかをテーマに研究をつづけているが、今回はその一環として精子の微細構造についてのべる。

9月上旬にはすでに貯精嚢に完成した多数の精子が含まれている。これらを材料とし定法通り試料をつくり観察に供した。材料の採集に際し貯精嚢を切開したが、この時、嚢の外に塊りとなって出た精子も観察した。貯精嚢に残っていた精子と違いはなかった。

精子は頭部(先体あり)、頸部、尾部から成る細長い紡錘型をしている。先体の長さは $20\mu\text{m}$ 以上に及び、その巾は先端の 80nm から順次太くなり最大 $0.3\mu\text{m}$ に達する。先体の表面には 10nm の巾で全域に糖衣様の物質が存在しており、これによって、 $400\sim 500$ 匹の精子が頭部先端で相互に接着させられているように見える。貯精嚢内にあるのは上皮細胞の微絨毛とこの物質を介して接している。さらに中には 8nm の周期を示す縞状構造がみられる。

先体はacrosome spaceを介して核に連続している。

核は長さ $60\mu\text{m}$ 以上で、巾は最大 $0.36\mu\text{m}$ になる紡錘型で高電子密度の物質からできている。核の中に巾 $0.4\sim 0.46\text{nm}$ の、より電子密度の高い部分があり、縦断像では長軸方向に平行に走る線状にみえる。核後半部は中程度の電子密度の物質(Nuclear mantle)によってつまれながら、中片相当域(Centriole adjunct)のところまで細長く伸びて終る。Centriole adjunct部はおよそ $1\mu\text{m}$ あり、electron transparent space、中程度の電子密度の部分、Nuclear mantleの後端部、軸繊維よりなる。中心子は認められていない。

尾の基部は典型的な $9+9+2$ 型の軸繊維の配列を示す。その周辺にミトコンドリア由来の構造があり、その中にはelectron transparent spaceがみられる。尾の巾は最大 $0.45\mu\text{m}$ にも達し、末端になるにつれ、細くなり数本の軸繊維がみられるだけである。

EXPERIMENTS ON THE EMBRYONIC PHYSIOLOGY OF THE BIG
HEADED GRASSHOPPER, AULOCARA ELLIOTTI (Thomas):
ORTHOPTERA, ACRIDIDAE

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INTRODUCTION

The big headed grasshoper, Aulocara elliotti, inhabits rangelands and prairies of the Western United States and Canada and is considered among the most important pest species of these areas because it feeds upon a native wheat grass, Agropyron smithii, a perennial species fed upon by domestic cattle. A. elliotti is monovoltine, having but one generation per year, with the egg stage overwintering in obligatory diapause. The sexes are dimorphic, the male being slightly smaller than the female. Hatching from the egg generally occurs after mid-April and there are five nymphal instars which develop during May and June. The adult molt may take place during the last week of June or as late as the end of July depending upon the climatic conditions of the year.

My research, which is sponsored by the Montana Agricultural Experiment Station, has been concerned with various aspects of the embryology of A. elliotti, initially with developing a staging criteria for the external morphogenesis and with study of the histology of embryogenesis. More recently my research has been concerned with factors, intrinsic and extrinsic, which affect the physiology of embryonic development. In this presentation I would like to tell you something about two aspects of my research: first, about experiments dealing with the effects of maternal environmental factors which affect the embryonic morphogenesis, and second, concerning experiments which give evidence for embryonic hormonal control of certain developmental events, in particular the embryonic diapause.

I. EFFECTS of MATERNAL FACTORS on EMBRYONIC DEVELOPMENT.

A. External Morphogenesis.

The external morphogenesis of A. elliotti was described in 27 discrete stages beginning with Stage 1 when a circular disc appears at the posterior or hydropyle end of the egg at about seven days at 25°C.

Pre-diapause development requires about 40 days at that temperature, with most embryos entering diapause at Stage 19. A survey of eggs collected from wild populations during the winter showed that many embryos overwinter in younger developmental stages. When these "retarded" embryos were sectioned and studied histologically, no mitotic figures were present indicating that that aspect of their development was similar to normal embryos which overwinter at Stage 19 in diapause. Embryos which fall behind the usual pattern of development also occurred in laboratory populations where temperatures have been optimal for their continued development. This finding led to investigations to learn the causes of such wide variations in the rate of embryonic development of this species.

B. Maternal Aging.

Since wide variation in the rate of embryonic development was found in embryos reared under identical conditions, it was speculated that perhaps there were factors transmitted to the embryo through the cytoplasm of the egg by the female parent which could affect the rate of embryonic growth. The effects of maternal aging were first to be investigated. Fifty pairs of nymphs were reared to adulthood and separated at the day of the imaginal molt into single pairs. The egg pods were collected daily and incubated to 30 days of age at 25°C. Eggs were fixed in Bouin's solution and stored in 70% ethanol until their embryos were staged and body length measurements taken according to criteria for this species (Van Horn, 1966a). When the stages of development of these sibling embryos were plotted against the ages of their female parents at the dates of laying, a linear relationship was found. Likewise, body lengths of embryos plotted against maternal age at the dates of laying gave similar curves and regression coefficients. Embryonic development was found to proceed at a linear rate of increase with the aging of the female parent until she reaches 35-40 days of age. At that time the rate of development of the embryonic progeny declines. The first eggs of 49 out of the 50 pairs of adults were sterile, and in some instances, also the last eggs laid failed to develop viable embryos. When the distribution frequency of stages of embryos were plotted, it was found that about 5% of the embryos attained each of the pre-diapause stages of development,

giving a linear pattern rather than a maximum frequency at Stages 12-14 usually found.

C. Adult Photoperiod and Rearing Density.

In the following year, a similar experiment was conducted with A. elliotti collected as nymphs from a population near Decker, Montana. In this study the parental adults were reared under controlled conditions of daylength at two different densities: either long (16 hours) or short (8 hours) daylengths, and with either one or two pairs per cage. When the distribution of stages of embryos were plotted with maternal age at the date of laying, significant differences were found in the rate of embryonic development between the different parental environmental rearing regimens. 30-day-old embryos from females reared under long day photoperiod with one pair per cage had a mean stage of 8. Those from parents reared at long days with two adult pairs per cage had a mean stage of 9.5; those with parents reared at short days, one pair per cage has a mean stage of 10 and those with parents reared under short day conditions with two adult pairs per cage had an average stage of 11. These data demonstrate that parental day lengths and density have important effects upon the rate of embryonic development of the offspring. The evolutionary advantage may be that embryos laid late in the fall under short day conditions will develop rapidly, reaching diapause in a shorter period of incubation than embryos laid at the beginning of the laying season when daylengths are long and there is ample time to develop to Stage 19 before winter conditions prevail.

D. Changes in Fecundity, Egg Viability and Rate of Development with Reduced Parental Rearing Temperature and with Population Source.

Because embryonic development in A. elliotti was found to be a sensitive indicator of parental environmental factors, it was decided that the numbers of eggs produced and their percentage of viability could perhaps be useful predictive agents for the vigor of wild populations of grasshoppers over a period of years. To this end, six wild populations of A. elliotti were chosen for study and from 1968 until 1975 a representative sample of 10 - 15 pairs of adults were reared as single pairs under two temperature regimens (18 - 24.5°C and 24.5 to 29°C). Their egg pods were collected daily, incubated to 30 days of age at 25°C, then fixed and their embryos staged and measured.

It was found that the lower temperature regimen caused greatly reduced fecundity and also reduced the percentage of viable eggs produced. It was learned that similar patterns of fecundity and viability were present in the populations from Townsend and Simms, although these are isolated from each other by approximately 75 miles of mountainous region, and that two other populations, those from the O.W. Ranch and Decker, likewise had similar patterns of reproduction and viability. Since all parental pairs were reared identically each of the eight years under laboratory conditions, changes in fecundity and viability which differed between the four populations had to result from differences present in the nymphal insects which carried through to their embryonic offspring. The mean stages and lengths of embryos from a given population were to found differ significantly from year to year and between populations in a given year. It was questioned whether there could be changes in the quality of the host food plant of the nymphs which could so influence the parental female that her reproductive performance might differ significantly from that of her offspring and that of their progeny. Trends of reduced viability and fecundity were observed over periods of several years in given populations, and those traits were strongly correlated within a given year.

E. Effects of Parental Host Plant Rearing Temperature on Embryonic Development.

An experiment was begun in 1976 to test the effects of the host plant rearing temperature upon the reproduction and viability of eggs of the grasshoppers feeding upon it. Two groups of 15 single pairs of A. elliotti were reared according to standard methods and fed grass from a field site near Bozeman as in previous years, with one under "hot" and the other under "cold" conditions as before. Other groups of 15 pairs each were fed grass transplanted from a field site near Red Bluff Experiment Station into the insectary and reared under the same "hot" and "cold" conditions as the adult grasshoppers. When the fecundity of adults reared in the "hot" environment and fed grass reared at different temperatures was compared, it was found that those grasshoppers fed grass raised in the "cold" environment laid significantly greater numbers of eggs than those fed either field grass or "hot" reared grass. And further, the viability of the eggs was

greater in groups fed grass reared in the "cold". One of the populations under study, that from Townsend, Montana, had females with shorter life-spans in the group fed "cold" reared grass. It was necessary, therefore, to compare the fecundity of the different feeding regimens in both populations by calculating the reproductive success in terms of the mean number of eggs laid per day of adult female lifespan. When these data were obtained it was found that females from Decker fed "cold" grass laid an average of 1.04 eggs per adult female day of lifespan, while those fed "hot" reared grass laid .65 eggs per day and those fed on field grass laid .71 eggs when all adults were reared in the "hot" environment. In the Townsend population females fed "cold" reared grass laid .64 eggs, those fed "hot" reared grass, .58 eggs, and those fed field grass laid .60 eggs per female adult day when adults were reared in the "hot" environment. When the adult females were reared in the "cold" environment, their reproduction was significantly reduced, yet the effect of feeding grass reared in the "cold" environment was still apparent, with females from Decker fed "cold" grass laying .24 eggs/female day while those fed the other grass laid .12 and .14 eggs respectively. The Townsend population reared in the "cold" environment responded similarly, though with less difference between feeding groups.

It is thought that factors other than those having usual nutritive significance are responsible for the effects observed, since total nitrogen analyses of the three different grasses revealed only 2% difference between the "hot" and "cold" reared grasses. Experiments are underway at present to determine the nature of the growth factors involved and whether they may be hormonally related.

F. Maternal Endocrine Gland Transplants.

Another experiment conducted earlier gives reason to believe that factors which alter the maternal endocrine system may significantly affect both fecundity and egg viability. The retrocerebral endocrine complex, including the corpora cardiaca-corpora allata and occipital ganglion, was transplanted from a reproductive adult donor into one-day old or 15-day-old adult female A. elliotti. Those females receiving transplants were maintained according to standard methods, their eggs collected daily, reared to 30 days of

age, and their embryos then staged and measured. When the data pertaining to fecundity and viability were compared with the controls, both injected or untreated, it was learned that the transplants had enhanced the fecundity and viability only when added at 15 days of adult age. When injected at one day after the adult molt, the effect of the transplant was to reduce the fecundity and viability in a manner similar to that observed when grasshoppers were reared in the "cold" environment. The neuroendocrine basis for these findings will be investigated in the future.

II. EVIDENCES for EMBRYONIC HORMONAL CONTROL of DEVELOPMENTAL EVENTS.

Now I would like to mention experiments which have dealt with another aspect of my research, that of trying to obtain evidence for endocrine regulation of the embryonic development by the embryo itself.

A. Gland Volume Changes during Extended Embryonic Diapause.

Histological studies revealed that the endocrine glands of A. ellioti appear at Stage 12 and undergo growth and maturation during the remainder of prediapause development. Sections of an embryo at Stage 17 showed the ventral head glands to be well formed at either side of the embryo, two stages prior to the diapause Stage 19. Since there has been much discussion and controversy about whether growth and development takes place during obligatory embryonic diapause, I incubated some diapausing embryos for different periods, with and without intervals of low temperature treatment and then sectioned them to compare with embryos just entering the diapause. A section of the ventral head glands of an embryo 240 days of age at Stage 19 in diapause shows that they are considerably enlarged, that the embryonic hemolymph is stained more darkly and that other tissues appear to have undergone growth in size. To determine whether increases also occurred in different endocrine organs, a series of embryos were sectioned and their gland volumes determined by making planimeter measurements of camera lucida drawings of every sections of each gland. The results show that there are gradual increases in the volumes of the corpora allata, corpora cardiaca and the ventral

head glands (prothoracic gland homologues) with the length of diapause. Further, it was found that embryos treated with juvenile hormone analogue had glands with the largest volumes.

B. Diapause Termination with Juvenile Hormone Analogue.

A series of embryos were treated with a geranyl ethyl ether compound, (Stauffer, Inc.) known to be an analogue of juvenile hormone, to determine the effect upon the diapause of embryos previously chilled which had failed to terminate that condition. Nine out of ten embryos immediately underwent blastokinesis, signalling termination of diapause when they were painted directly over the brain with JH analogue. Embryos treated by immersion in a solution of JH in methanol likewise terminated diapause in a significant number of instances. The embryos which terminated diapause immediately began to develop pigmentation and sclerotization similar to that observed in definitive embryos. This process, however, occurred so rapidly that normal yolk engulfment was not accomplished and the embryogenesis could not be normally completed.

Novak (1969) has referred to the effects of JH analogues on grasshopper embryogenesis in Schistocerca gregaria as causing retardation in development. I believe, on the contrary, that the effects brought about by JH analogue should be thought of as representing precocious development, since the embryo rapidly takes on characteristics similar to those of normal embryos just prior to hatching.

It seems likely that the cause of precocious sclerotization and of failure to undergo yolk engulfment and dorsal closure is the result of extraordinary physiological stimulus rendered by the JH analogue used. The compound used is known to have activity far exceeding that of natural C16 JH.

C. Effects of Corpora Allata Transplants in Developing Embryos.

To test the effects of natural JH upon one aspect of embryonic development, pigmentation and sclerotization of the exoskeleton, another group of gland transplants were made, this time using corpora allata from first instar nymphal grasshoppers to provide the hormone source. Embryos which had just completed blastokinesis were chosen as recipients for the corpora allata transplants.

Egg membranes covering the embryo were carefully removed making certain not to injure the yolk sac. A single corpus allatum was dissected from the donor nymph and injected into the lateral wall of the first abdominal segment. Embryos were then suspending in physiological saline solution and inverted into hanging drops. Preparations were sealed with vaseline jelly and placed at 25°C for incubation. Within two to four weeks the effects of the implanted corpora allata were visible. On the operated side, that receiving the corpus allatum, the embryonic cuticle remained unpigmented and unsclerotized, revealing clearly the yolk mass through the abdominal body wall, while the opposite untreated side showed normal pigmentation and sclerotization taking place. It was concluded that the processes of sclerotization and pigmentation, known to be under the control of the hormone ecdysone, are prevented by injection of glands producing juvenile hormone, and thus that the response of developing embryonic tissues to physiologically normal amounts of that hormone are similar to that observed in nymphal stages of development with similar experimental procedures in other insect species.

SUMMARY OF CONCLUSIONS:

- I. A. There is wide variability in the rate of embryonic morphogenesis which is unrelated to the embryonic genotype or environment.
- B. The rate of embryonic development prior to diapause is highly correlated to the age of the female parent at the date the egg is laid.
- C. Parental daylength and rearing density significantly influence the rate of embryonic development.
- D. 1. Parental fecundity and viability of the eggs are correlated to the adult rearing temperature and population source.
2. Fecundity and viability and rate of embryonic development vary significantly within a given population from one year to the next when adults are reared under similar environmental conditions.
- E. The source and rearing temperature of the host plant may have significant effects upon the fecundity and egg viability of

the grasshopper adults feeding upon it.

F. Supernumerary retrocerebral endocrine transplants in parental females one day after the imaginal molt cause reduced fecundity and egg viability similar to that of unoperated controls reared in a "cold" environment. At 15 days after the imaginal molt, the same gland transplants stimulate increased fecundity.

II. A. Significant growth of the fat body, muscles and gonads occurred in embryos incubated for increasingly long periods in diapause.

The volumes of the corpora allata, corpora cardiaca and ventral head glands increased with the period of diapause incubation and responded significantly to application of juvenile hormone analogues.

B. The obligatory diapause of A. ellioti was terminated in a significant number of embryos following exposure to JH analogue. Subsequent development proceeded too rapidly for normal morphogenesis to occur, i.e. definitive sclerotization and pigmentation occurred in embryos just completing blastokinesis and treated embryos, therefore, failed to engulf their yolks or to complete dorsal closure.

C. Transplants of corpora allata from nymphs of A. ellioti into postdiapause embryos brought about normal inhibition of cuticular sclerotization and pigmentation, similar to that observed in nymphal insects in other species.

EMBRYONIC DEVELOPMENT OF LARVAL EYES OF PANORPA
PRYERI MACLACHLAN (MECOPTERA)

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It was observed that the process of the embryonic development of larval eyes of Panorpa pryeri MacLachlan is fundamentally similar to the compound eye formation in other insects and differs entirely from the lateral ocellar formation in holometabolous insects.

In general the newly hatched nymphs of hemimetabolous insects already have well developed compound eyes. The larvae of holometabolous insects, in contrast to hemimetabolous nymphs, generally have lateral ocelli or stemmata usually composed of six ocelli and only the adult insects have compound eyes. Mecopteran scorpionflies, however, have well developed compound eyes in the larval stage, so that they are a rare exception in holometabolous insects.

The fact that the larvae of Panorpa have well developed compound eyes though they bore tunnels just beneath the surface of the soil and live in them is significant when examining the phylogenetical characteristic of the Mecoptera.

VISUAL ORGAN OF GALLOISIANA NIPPONENSIS

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Fine structure of the lateral eye of G. nipponensis, the commonest species of Grylloblattodea in Japan, was observed with the scanning and transmission electron microscope.

The eye is composed of thirty eight ommatidia roughly aggregated in an ellipsoid area. Surface of eye region is covered with arabesque secretions presumably waxy substance which arose from the pits closely distributed on the surface of cuticle. Although the cornea forms a biconvex lens in cross section, it seemed to be less specialized as lens. Because the pore canals are present as in common cuticle. Though the number of reticula cell in an ommatidia is variable,

twelve cells are commonly found. These cells are arranged in two segment in upper and lower position, each of which bears rhabdome and forms the fused type rhabdom. Rhabdomic microvilli are irregularly arranged and become vacuole or lysosomal structure in proximal part.

There is no differentiation in pigment cells into the primary- and secondary ones.

ON THE EMBRYONIC DEVELOPMENT OF PROTOHERMES
GRANDIS THUNBERG (MEGALOPTERA, CORYDALIDAE).---
CHANGE IN EXTERNAL FORM OF THE EMBRYO

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Egg cylindrical with somewhat convex ventral side (1.25 mm x 0.53 mm) with conspicuous micropylar apparatus at the anterior pole. Embryonic period is 13 days. The germ band, from the beginning, occupies most of the ventral midline of the yolk surface. The completion of the embryonic membranes is preceded by the metamerism in the gnatho-thoracic region. The revolution of the embryo begins first at the posterior end of the abdomen. Embryonic ecdysis occurs after the completion of the thoracic legs. The embryonic features closely resemble those of Sialis lutaria L. and in some extent those of Chrysopa perla L. The mode of the revolution of the embryo resembles that of trichopteran and siphonapteran embryos.

PRODUCTION OF 'DOUBLE CEPHALON' MALFORMATION BY
THE UV IRRADIATION TO THE POSTERIOR END OF THE EM-
BRYO AT THE EARLY DEVELOPMENTAL STAGES OF
CHIRONOMUS SAMOENSIS.

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In a previous study, the author obtained two types of double malformation, 'Double cephalon' and 'Double abdomen', by partial irradiation of UV light to the posterior and anterior half of

Chironomus egg, respectively (Yajima, 1964). The frequency of occurrence of the malformations, however, was rather low: 5.2% (double cephalon) and 22.2% (double abdomen) at their maximum rates.

Later, Kalthoff and Sander (1968) succeeded in producing the 'double abdomen' in Smittia spec. (Chironomidae, Diptera) after Yajima's procedure. Further, in the succeeding studies ('69 - '77), they got the following interesting results ; (1) high frequency of double abdomens can be obtained after the incubation of the UV-irradiated eggs at lower temperature (8°C), while the frequency is low after the incubation at higher temp. (24°C). This phenomenon was named as "Thermoreversion" by Kalthoff. (2) the monsters can be obtained from early pre-pole cell stages to later pre-blastoderm stages. (3) the effective target for double abdomen formation is extranuclear and are localized mainly in the superficial layer of egg before anterior 1/4 the egg length. (4) the action spectrum for double abdomen induction has a peak at about 280 nm with a shoulder at about 260 nm and the treatment of the egg by RNase also causes the formation of double abdomen, suggesting that the first candidate for the target must be RNP. (5) However, as repeatedly stated by him, his attempts to reproduce double cephalon by UV irradiation of Smittia and Chironomus tentans eggs have completely failed so far. As a result, his hypothetical model to explain the establishment of segment pattern of Smittia was somewhat defective (Kalthoff, '76).

In the present study, the author succeeded in obtaining the double cephalons in a high frequency in suitable experimental conditions as follows: the malformations can be obtained within narrow range of developmental stage laing between 10' and 30' minutes after nuclear migration; the UV irradiation from the posterior side to the posterior end of egg is effective for the production of the monsters; the higher frequency can be obtained at lower temperature incubation (20°C) ("Thermoreversion"). The formation of double cephalon is photoreversible. Since these conditions concerning the production of double cephalon are almost the same as those shown in the production of double abdomen of Smittia by Kalthoff, both the effective targets for the double cephalon double abdomen may have common natures.

But, there is a little difference in reaction to the experimental procedures between the two malformations. The difference may have relation to the determination of either cephalic or abdominal characters.

A CYTOPLASMIC FRACTION FROM DROSOPHILA EMBRYOS, CAPABLE OF INDUCING POLE CELLS.

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Homogenate of 10^6 Drosophila embryos at early nuclear multiplication stage was fractionated to obtain a fraction including factors to induce pole cell formation on the embryos previously inabled for the self-generating pole cell formation by UV irradiation at the posterior pole. The fractionation was undertaken by differential centrifugation and by centrifugation on a discontinuous sucrose density gradient.

A fraction, little lighter than the mitochondrial fraction, was most effective in inducing pole cell formation. The pole cell inducing activity was little reduced even if the fraction was lyophilized or if the embryos were frozed for a week at -20°C preceding to the homogenization. The lyophilized "active" fraction induced pole cell formation either at posterior pole or at anterior pole of the embryos according to the place where the fraction was deposited by injection.

One of the grandchildless-type mutants which we induced by application of EMS was rescuable by the injuction of our obtained lyophilized active fraction.

The pole cells induced by the lyophilized active fraction were found to include morphologically normal polar granules. The active fraction was elctron microscopically checked to include a number of polar granules and ribosomes.

EXPERIMENTALLY INDUCED SUPERNUMERARY SEGMENT IN THE HORSESHOE CRAB

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The monster having supernumerary segments was induced by the treatment with NaHCO_3 , Ca^{++} free sea water or Actinomycin D.

Normal horseshoe crab has six prosomal segments. This monster had a or two excess segments behind the 6th prosomal segment. The appendages of the excess segment had the same form and structure as the 6th prosomal appendages which are biramous.

This monster was formed by the treatment at the developmental stage just before the appearance of segments.

The effective treatment time was 24 hours.

The most effective concentration was 10^{-1}M in case of NaHCO_3 and $5\ \mu\text{g}/\text{ml}$ in case of Actinomycin D. In case of Ca^{++} free sea water, the monster was formed at the highest rate by the treatment with the Ca^{++} free sea water containing $10^{-3}\ \text{M}$ EGTA.

The induction of this monster with NaHCO_3 may be due to the combination CO_3^{--} ion in NaHCO_3 with Ca^{++} ion in the embryo.

ELECTRON MICROSCOPICAL OBSERVATION OF EARLY EMBRYONIC DEVELOPMENT OF THE SILKWORM BOMBYX MORI

I. STRUCTURES OF NEWLY LAID EGGS AND CHANGES BY SPERM ENTRY

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Structures of newly laid eggs of the silkworm, such as chorion, vitelline membrane, "inter membranous layer", and periplasm, were observed electron microscopically.

Organelles in the periplasm, especially structure and/or distribution of mitochondria, endoplasmic reticulum, and ribosomes, were remarkably different in the different egg regions. The first changes by sperm entry appeared in the vitelline membrane and intermembranous layer and then in the periplasm.

The results suggest a possibility of solving relation between sperm entry and egg surface, and regional difference of periplasm and cell differentiation at the blastoderm stage.

ULTRASTRUCTURE OF SPERMATOOA OF THE MANTIS,
TENODERA ARIDIFOLIA (DICTYOPTERA: MANTODEA: MANTIDAE)

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Ultrastructure of the spermatozoa of *Tenodera aridifolia* was reported. Structural differences as compared to spermatozoa of other insects are as follows: localization of an electron dense material on the surface of acrosome, existence of periodic striations of 8 nm in the acrosome, and a nuclear mantle covering the distal part of nucleus.