BARD

FINAL REPORT

PROJECT NO. IS-2403-94

**Biochemical and Molecular Analysis of Juvenile
Hormone Synthesis and its Regulation in the
Mediterranean Fruit Fly (*Ceratitis capitata*)**

S.W. Applebaum, L.I. Gilbert, D. Segal

2000

BARD Final Scientific Report
(Cover Page)

Date of Submission of the report: February 21, 2000

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Project Title: Biochemical and Molecular Analysis of Juvenile Hormone Synthesis and Its Regulation in the Mediterranean Fruit Fly (*Ceratitis capitata*)

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Continuation of (Related to) Previous BARD Project: Yes

Number: IS-1664-89

Keywords *not* appearing in the title and in order of importance. Medfly, allatal maturation, allatostatin, reproduction, sex peptide, *apterous*, *Drosophila*, epoxidase inhibitors

Abbreviations used in the report, in alphabetical order: CA = corpus allatum; FR-7 = (fraction #7 - unknown JH homolog); HPLC = high performance liquid chromatography; JH - juvenile hormone; JHB₃ = juvenile hormone III bisepoxide; SP = sex peptide; TLC = thin layer chromatography.

Budget: IS: \$240,000

US: \$60,000

Total: \$300,000

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Principal Investigator

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Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted)				
Submitted, in review, in preparation	1			
Invited review papers				
Book chapters				
Books				
Master theses				
Ph.D. theses				
Abstracts	2			
Not refereed (proceedings, reports, etc.)				

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted				
Issued (allowed)				
Licensed				

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Visits/Meetings	2	1		
Sabbaticals				
Postdoctorates				

- **Cooperation**, briefly explain whether synergistic, complementary or supportive.

The studies in Rehovot and Chapel Hill were supportive: the emphasis in both laboratories is on insect biochemistry and molecular biology. the emphasis in Tel-Aviv is on molecular genetics, and their contribution was therefore complementary.

Abstract

Original Objectives and revisions: (1) "To determine the biosynthetic pathway of JHB₃ in the adult *C. capitata* CA in order to establish parameters for the future choice and synthesis of suitable inhibitors". Modified: to determine the pattern of FR-7 biosynthesis during normal reproductive maturation, and identify enzymes potentially involved in its synthesis. (2) "To correlate allatal epoxidase activity to the biosynthesis of JHB₃ at different stages of reproductive maturation/vitellogenesis and evaluate the hypothesis that a specific JH-epoxidase may be rate limiting". Modified: to study the effects of epoxidase inhibitors on the pattern of allatal JH biosynthesis in vitro and on female reproduction in vivo. (3) "To probe and clone the gene homologous to *ap* from *C. capitata*, determine its exon-intron organization, sequence it and demonstrate its spatial and temporal expression in larvae, pupae and adults."

The "Medfly" (*Ceratitidis capitata*) is a serious polyphagous fruit pest, widely distributed in subtropical regions. Damage is caused by oviposition and subsequent development of larvae. JH's are dominant gonadotropic factors in insects. In the higher Diptera, to which the Medfly belongs, JHB₃ is a major homolog. It comprises 95% of the total JH produced in vitro in *D. melanogaster*, with JH-III found as a minor component. The biosynthesis of both JH-III and JHB₃ is dependent on epoxidation of double bonds in the JH molecule. The specificity of such epoxidases is unknown. The male accessory gland *D. melanogaster* produces a Sex Peptide, transferred to the female during copulation. SP reduces female receptivity while activating specific JH biosynthesis in vitro and inducing oviposition in vivo. It also reduces pheromone production and activates CA of the moth *Helicoverpa armigera*. In a previous study, mutants of the *apterous (ap)* gene of *D. melanogaster* were analyzed. This gene induces previtellogenic arrest which can be rescued by external application of JH. Considerable progress has been made in recombinant DNA technology of the Medfly. When fully operative, it might be possible to effectively transfer *D. melanogaster* endocrine gene-lesions into the Medfly as a strategy for their genetic control.

A marked heterogeneity in the pattern of JH homologs produced by Medfly CA was observed. Contrary to the anticipated biosynthesis of JHB₃, significant amounts of an unknown JH-like compound, of unknown structure and provisionally termed **FR-7**, were produced, in addition to significant amounts of JH-III and JHB₃. Inhibitors of monooxygenases, devised for their effects on ecdysteroid biosynthesis, affect Medfly JH biosynthesis but do not reduce egg deposition. FR-7 was isolated from incubation media of Medfly CA and examined by various MS procedures, but its structure is not yet resolved. MS analysis is being done in collaboration with Professor R.R.W. Rickards of the Australian National University in Canberra, Australia. A homologue of the *ap* gene of *D. melanogaster* exists in the Medfly. LIM domains and the homeo-domain, important for the function of the *D. melanogaster ap* gene, are conserved here too. Attempts to clone the complete gene were unsuccessful.

Due to the complexity of JH homologs, presence of related FR-7 in the biosynthetic products of Medfly CA and lack of reduction in eggs deposited in the presence of monooxygenase inhibitors, inhibition of epoxidases is not a feasible alternative to control Medfly reproduction, and raises questions which cannot be resolved within the current dogma of hormonal control of reproduction in Diptera. The Medfly *ap* gene has similar domains to the *D. melanogaster ap* gene. Although mutant *ap* genes are involved in JH deficiency, *ap* is a questionable candidate for an endocrine lesion, especially since the *D. melanogaster* gene functions is a transcription factor.

Achievements

Significance of main scientific achievements

The JH homologs produced in the adult Medfly CA are JHB₃, JH-III and an unidentified hexane-soluble component (FR-7). The chemical identity of this compound is unclear at this time. We conclude tentatively that FR-7 is a methyl ester prior to farnesoic acid, the latter being the penultimate precursor of JH-III. There are several indications that FR-7 may be significant for reproduction. Thus, its biosynthesis is enhanced by the heterologous *D. melanogaster* sex peptide, which is known, in other insect systems, to enhance allatal JH production. Additionally, its temporal appearance coincides with competence of the CA to produce JH in vitro, and with oogenesis and egg deposition by the gravid female.

Inhibitors of epoxidation of the C_(6,7) double bond (unique to JHB₃) do not affect oviposition, suggesting that high concentrations of JHB₃, the major JH homolog in the Medfly as in all other higher Diptera studied to date, may not be required for reproduction.

Potential for application and estimated economic benefits

We originally hypothesized that inhibition of epoxidation of JHB₃-precursor at the C_(6,7) double bond might lead to the development of a novel method for the control of the Medfly. The absolute amounts of FR-7 produced in vitro by Medfly CA are insufficient for determining bioactivity. Therefore, in order to conclude whether and how FR-7 is significant in Medfly reproduction, and might therefore be exploited in the broad context of Medfly control, we are presently collaborating on a study of its chemical identity, in order to synthesize the compound and determine its bioactivity. The economic benefits of this project could potentially be high.

Estimated impact

The impact of this project on crop yield or on saving of inputs is unclear, based on the considerations detailed above.

Other benefits

- (1) Although the structure of FR-7 is still unresolved, it is an endogenous product of the Medfly CA, which incorporates the methyl moiety of methionine and may have significance individually, or within the array of JH homologs produced by

Medfly CA. If it should prove to have pronounced physiological significance on Medfly reproduction, this information could then be exploited for devising specific ways to interfere with the damage imposed by this important economic pest.

- (2) The cross reactivity of *D. melanogaster* SP in the Medfly system raises the possibility that SP-transformed sterile Medfly males can be constructed as target-oriented vectors of reproductive control, the target being the conspecific females, and they only. By using infertile male mutants, there would be no concern of possible gene transfer into the native population of insects. This could improve the Sterile Insect Technique, providing that it results in suppression of reproduction and that the induced changes in JH profile are beneficial.

Details of cooperation

In addition to standard lines of communication between the three laboratories, such as e-mail, fax and telephone discussions, the principal investigators and associates met periodically and P. Moshitzky, one of the associates from the Rehovot group, travelled to Chapel Hill and worked in L. Gilberts laboratory during Nov-Dec 1998. Epoxidase inhibitors were selected and sent from Chapel Hill to Rehovot at an early stage of the project. The principal investigators and associates actively participated in the 5th International Conference on Invertebrate Neurochemistry and Neurophysiology, which convened in Eilat in September 1997, and in the 7th International Conference on the Juvenile Hormones, which convened in Jerusalem in August 1999 and discussed the progress of this research. At that conference we also met with Professor R.R.W. Rickards, of the Australian National University in Canberra, with whom we are collaborating in the determination of the chemical identity of FR-7. Dr. Yoram Roessler of the Citrus Marketing Board of Israel kindly supplied us with pupae of their "Wild-type" strain of *C. capitata*, originating from a local Israeli population cultured routinely in their mass-rearing facilities at the Biological Control Laboratories and supplemented annually with field material.

Body of the Report

Background

The hazard chemical insecticides pose to humans, livestock and the environment serve as a strong impetus for developing alternative means for pest control. Disrupting regulatory processes of reproduction might be an alternative strategy for controlling population increase of insect pests.

The Mediterranean fruit fly, popularly termed the "Medfly" (*Ceratitis capitata*), is a serious polyphagous pest of numerous fruit crops and is widely distributed in subtropical areas of the world. More than 250 botanical species are regarded as potential hosts worldwide. It often causes major crop loss in many developing countries, and is a major "qualitative" pest in developed countries. The term "qualitative" is meant to describe a situation where even very low levels of infestation impose restrictions on trade. The Medfly produces 8-9 annual generations in the coastal plain of Israel, bridging a great variety of fruits. It is firmly established in Mediterranean countries and in the state of Hawaii. The question of whether it is chronically present in citrus producing areas in the continental United States, or is occasionally introduced, has been the subject of some debate (Carey, 1992). There is no question that attempts to completely eradicate this potentially dangerous pest of the citrus industry, following periodic outbreaks in the United States, are very costly.

Juvenile hormones (JHs) are primary regulators of development and reproduction in insects. They participate in the regulation of the orderly and timely transition of developmental stages and are major gonadotropic hormones in the adult (Doane, 1973). JH regulates the synthesis of yolk proteins in the female fat body and ovary and their uptake into the oocyte (Koeppel et al., 1985). It mediates the onset of mature sexual behavior in both males and females (Gadenne, 1993; Duportets et al., 1996; Pener and Shalom 1987; Schal et al., 1997).

Various JH compounds are produced in different proportions in different dipteran species. In the higher Diptera, the predominant JH species produced in vitro by the CA is JHB₃. In *Drosophila melanogaster*, JHB₃ comprises some 95% of the total (Richard et al., 1989; Altaratz et al., 1991, Moshitzky and Applebaum, 1995), the remainder being JH-III. In *Phormia regina*, more appreciable amounts of JH-III may be produced (Liu et al., 1988; Zou et al., 1989; Yin 1995). Allatal activity is down-regulated in *Drosophila* by cerebral allatostatic neuropeptides and by direct

axonal control, separately controlling the biosynthetic activity of the gland at apparently different sites in the biosynthetic pathway of JH (Moshitzky and Applebaum, 1995). We had previously identified JHB₃, a novel form of JH in flies, which has **two** epoxide groups, and differs in this respect from all other JH homologs in insects, which contain a **single** epoxide group. This raised the possibility that JHB₃ might have specific significance in flies and led us to study and identify characteristics of its synthesis by the endocrine corpora allata (CA) glands and its cerebral regulation. The physiological significance of the dominant production of the unique JHB₃ in the higher Diptera is still unclear. A specific function for JHB₃ is possible, in which case its production by the Medfly CA might then be a promising target for control of this major pest. This reasoning led to this present study of JH production by the adult Medfly CA and of its regulation, whether cerebral or male derived.

The postmating decrease of receptivity of the mated females is induced by secretory peptides produced by the male accessory gland epithelium (see reviews by Chen, 1984; Gillott, 1988; 1996). These compounds are transferred in the seminal fluid to the female during copulation. Some of the accessory gland peptides pass through the vaginal wall into the haemolymph (Monsma et al., 1990), and gain access to their presumptive target tissues, the nervous and/or the endocrine system of the female. In the fruitfly *Drosophila melanogaster*, a 36 amino acid Sex Peptide (SP) is produced in the male accessory glands. It has been isolated and characterized (Chen et al., 1988).

1	10	
trp-glu-trp-pro-trp-asn-arg-lys-hypro-thr-		
11	20	
-lys-phe-hypro-ile-hypro-ser-hypro-asn-hypro-arg-		
21	24	36
-asp-lys-trp-cys-arg-leu-asn-leu-gly-pro-ala-trp-gly-gly-arg-cys		

Amino acid sequence of *D. melanogaster* Sex Peptide

Injection of synthetic SP, and heat-induced ectopic expression of SP in transgenic virgin females, elicit typical post-mating reactions rapidly (Aigaki et al., 1991; Schmidt et al., 1993a). After a normal mating encounter, female non-receptivity persists for about 7-9 days. In addition, JH biosynthesis is enhanced, presumably to stimulate oogenesis (Moshitzky et al., 1996). We have recently shown that *D. melanogaster* SP cross-reacts in a moth system (Fan et al., 1999). This suggests that this scheme of behavioural regulation of sexual activity by bioactive male peptides may be widespread in insects. This conclusion led us to examine the effect of *D. melanogaster* SP in the Medfly system as well.

Genetic and molecular studies of *C. capitata* are still far behind the remarkable achievements achieved in the analysis of *D. melanogaster*. Nevertheless, considerable progress has been made. Cytogenetic maps have been constructed (Zacharopoulou, 1990) and increasing numbers of Medfly genes are being isolated and characterized (e.g., Rosetto et al., 1997; Manzouridis and Fragoulis, 1998; Papadimitriou et al., 1998; Thymianou et al., 1998; Gagou et al., 1999).

Progress has been made in non-Drosophilid insects in recombinant DNA technology (Handler and O'Brochta, 1991; Handler et al., 1993; O'Brochta and Atkinson, 1996). This will facilitate the transfer of *D. melanogaster* endocrine gene-lesions into target insects as a strategy for their genetic control. We have previously analyzed mutants of the *apterous* (*ap*) gene of *D. melanogaster*. The *ap* gene is thought to be implicated in the JH system because the previtellogenic arrest typical of *ap*⁴ homozygous females can be alleviated by external application of JH. This indicates that the mutant ovaries are capable of normal response to JH, but that these flies presumably suffer some deficiency of JH (Postlethwait et al., 1976), perhaps due to a lesion in the regulation of its synthesis in the corpus allatum (CA). Homozygotes for the non-vitellogenic *Drosophila ap*⁴ mutation produce absolute levels of total JH as little as 6-fold less than the wild type level in adults, and about half of the wild type level in larvae (Altaratz et al., 1991). This is a chronic effect rather than a delay in the onset of JH production. The low level of total JH in the mutant is associated with a decrease in the proportion of JHB₃ and an increase in that of JH III and methyl farnesoate, suggesting that the activity or availability of the terminal epoxidases in the JH

biosynthetic pathway is reduced in *ap* mutants (Altaratz et al., 1991). The *ap*⁴ mutant CA apparently has normal size and gross morphology. However, EM studies have revealed features typical of inactive glands, namely low concentration of both smooth endoplasmic reticulum, mitochondria and mitochondrion-scalariform junction complexes (Dai and Gilbert, 1993). We have previously demonstrated that the *ap* gene encodes a transcription factor, which may regulate the expression of genes encoding allatostatic neuropeptides in the brain. We have found that certain aspects of JH-regulated reproductive behavior are aberrant in *ap* mutants (e.g., Ringo et al., 1991; Ringo et al., 1992; Cohen et al., 1992; Shtorch et al., 1995).

Methodologies and Materials

Insects

Experiments were performed with a “Wild-type” (WT) strain of *C. capitata*, originating from a local Israeli population cultured in the mass-rearing facilities of Biological Control Laboratories of the Citrus Marketing Board of Israel. Pupae were kindly supplied to us by its director, Dr. Yoram Roessler. Pupae were kept in Rehovot at a constant temperature of 25°C in a temperature and photoperiodic rearing chamber (12:12, L:D). Following adult eclosion, they were separated and crowded with sibling adults of the same sex in 2 liter clear plastic containers. In experiments where the effect of mating on allatal maturation and activity were examined, mature naive males and virgin female adults were brought together to mate for a measured time in large plastic jars. Adult flies were fed on a standard diet of bran, sugar and yeast.

JH biosynthesis in vitro

The bioassay is modified from Pratt and Tobe (1974) and is based on the incorporation of the methyl moiety of [³H-*methyl*]-methionine into JH (Moshitzky and Applebaum, 1995). Excised individual adult female CA-CC complexes were incubated for 4 h, unless otherwise mentioned, in 50 microlitre of Minimal Essential Medium (MEM) - without methionine or bicarbonate, containing 2% Ficoll 400™ and 50 mM HEPES buffer, pH 7.2, which was supplemented with 5microCi of L-[³H-*methyl*]- methionine (specific activity of 80Ci/mmol) (Amersham, U.K.) and 5X10⁻⁷M 3-octylthio-1,1,1-trifluoro-2-propanone (OTFP) as JH-esterase inhibitor (Hammock et al., 1984). At the end of the incubation period, the medium and tissue were extracted together with 150 microliter ice-cold hexane. Radiolabeled methyl

moiety incorporated into total hexane-soluble extract was determined by counting a 50 μ l aliquot in a β -counter (1209 RackBeta; LKB). Another 50 microliter aliquot was applied to silica gel plates (Polygram SIL G/UV₂₅₄; Macherey-Nagel) and JH-like compounds separated by development of the plates in hexane:ethyl acetate (3:2). Precursors were identified by short UV fluorescence of marker compounds and counted in a β -counter, using unlabeled JH-III and JH-III bisepoxide (JHB₃) as markers. Routinely, unless otherwise stated, 10 replicates were performed of each treatment and controls, and experiments were repeated 3 times on different dates. Values of incorporation are expressed as cpm/gland JH biosynthesis $\pm$ standard error.

Cerebral regulation of allatal activity

Excised CA-CC complexes, with axons to the brain intact, were incubated in vitro and the products analyzed by the above procedure.

Results

Developmental maturation of JH biosynthesis and its cerebral control in virgin and mated Medfly females

Newly emerged female Medflies are reproductively immature during the first few days after eclosion. A small percentage are able to mate two days later, and essentially all, within 4 days. JH biosynthesis parallels reproductive maturation: Basal levels of JH are produced in vitro by CA of newly emerged virgin females during the first three days after adult eclosion. Synthesis subsequently increases and levels peak at D-5 (Figure 1).

Figure 1: Allatal maturation in virgin and mated Medflies

When CA preparations were incubated with axonal connections to the brain intact, allatostatic control of total JH production in vitro was temporally established one day earlier in mated females, and is thereafter maintained at levels comparable to those observed in virgin females (Figure 2).

Figure 2: Cerebral allatostatic control of Medfly CA

We next examined the identity of the JH compounds produced in vitro. JHB₃ is the major component of the total JH produced in vitro by Medfly CA. In addition, significant amounts of JH-III and an unidentified JH metabolite, designated FR-7, are produced (Figure 3).

Figure 3: HPLC (left) and TLC (right) of radiolabeled products of virgin female Medfly CA

In order to evaluate the significance of FR-7 in the reproductive physiology of adult female Medflies, we questioned whether allatostatic regulation might be differentially reflected in the proportions of the different components produced during

allatal maturation. We found that allatostatic regulation of the three JH products of Medfly CA is dependent on the state of allatal maturation. Essentially, JHB₃ production in mated females parallels the pattern of developmental time-course observed for total JH production by Medfly CA (see Figure 1). Cerebral inhibition is most pronounced in days 4-6. The temporal pattern of JH-III production and its cerebral inhibition are similar. However, levels of FR-7 are **not** significantly reduced when CA are incubated with axonal connections to the brain intact (Figure 4).

Figure 4: Time-dependence of effect of axonal connection to the brain on in vitro biosynthesis of JHB₃ (top), JH-III (middle) and FR-7 (bottom) by virgin Medfly CA

Medfly CA were incubated for up to 8 h and the JH homologs separated by TLC and counted. Each time point was composed of different CA preparations. The time course of in vitro FR-7 biosynthesis in virgin females differs significantly from the biosynthesis of other JH components. Whereas, JHB₃ synthesis peaked after a 6 h incubation period and JH-III synthesis reached a constant level fairly rapidly, FR-7 synthesis increased linearly during the 8 h incubation period examined (Figure 5).

Figure 5: Incubation time-course of JH homolog biosynthesis by virgin female Medfly CA

There are several possible explanations for this. One is that FR-7 is not an endogenous CA metabolite, but might be a product of JHB₃ metabolism after its secretion into the incubation medium. We ruled out this possibility by incubating Medfly CA for 4 h in media lacking radiolabelled methionine, but supplemented with authentic radiolabelled JHB₃. No exogenous metabolism of JHB₃ to FR-7 was evident (data not shown). FR-7 production is not significantly stimulated by farnesoic acid, the penultimate precursor of JH-III and JHB₃, while the biosynthesis of JHB₃ and JH-III were (Figure 6).

Figure 6: Effect of farnesoic acid on production of JH homologs by virgin female Medfly CA

Effect of SP on the levels of JH homologs produced in vitro

In contrast to the stimulatory effect of SP on *D. melanogaster* JHB₃ biosynthesis (Moshitzky et al., 1996), 1 picomole SP depressed JHB₃ production in 1-2 day old virgin Medfly females, had no effect on JH-III production, but did significantly stimulate FR-7 production (Figure 7).

Figure 7: Effect of SP on in vitro production of JH homologs by virgin female Medfly CA

Effect of epoxidase inhibitors on the levels of JH homologs produced in vitro and oviposition in vivo

In order to select specific inhibitors of epoxidation appropriate for manipulation of JH biosynthesis in the Medfly CA, we examined two substituted imidazoles (KK-42 and TH-20) for their effect on JH epoxidation by *D. melanogaster* CA. Two candidate substituted imidazoles inhibitors were examined. The first, KK-42, has been proposed as an inhibitor of ecdysteroid synthesis in insects (Yamashita et al., 1987). Dilutions were prepared in acetonitrile, and 1 µl of the appropriate dilutions were added to the incubation media. Acetonitrile itself has no appreciable affect on the biosynthesis of JHB₃ by the *D. melanogaster* CA. The dose-responses to these two inhibitors is presented in the following two graphs (Figure 8).

Figure 8: Dose-response of virgin female *D. melanogaster* CA to KK-42 and TH-20 as expressed by in vitro production of JH homologs

JHB₃ is the major JH species produced in the control incubations, accompanied by a small amount of JH-III and a marginal amount of methyl farnesoate. This result is consistent with our previous studies (Richard et al., 1990; 1991). Both KK-42 and TH-20 markedly inhibit JHB₃ biosynthesis in a dose-dependent manner (Figure 8). There is a slight stimulation of JH-III and an appreciable stimulation of methyl farnesoate (MF) biosynthesis. Biosynthesis of JHB₃ proceeds in the adult female *D. melanogaster* CA from precursor farnesoic acid (FA) via epoxidation and terminal methylation (Moshitzky and Applebaum, 1995). It can be seen that the epoxidation at C_(10,11) (preceding JH-III biosynthesis) is partly inhibited at high concentrations by KK-42 and TH-20, while the epoxidation at C_(6,7) is markedly inhibited by both inhibitors. The increase in MF in the presence of TH-20 suggests that the specificity of this inhibitor for the position of the double bond is less strict.

KK-42 is more strict in specificity. Its effect on oviposition by virgin *D. melanogaster* females was therefore examined. One microliter aliquots of a KK-42 solution (10 microgram/microliter acetone) were topically applied on D-1 to the experimental females and 1 microliter of acetone alone, to the control females. Half of the experimental and control females were mated to naive males. The individual virgin females, or mated pairs, were kept individually in glass vials with artificial *Drosophila* medium, and the number of eggs deposited was noted 2 days later. KK-42 does not reduce the starting level of oviposition by either mated or virgin females (Table 1).

Table 1: Effect of KK-42 on cumulative oviposition over 2 days by virgin and mated *D. melanogaster* females

Treatment	Control (virgin)	Control (mated)	+KK-42 (virgin)	+KK-42 (mated)
Eggs deposited (+SE; n=20)	27±7	183±12	20±5	202±16

In contrast to its broad specificity in *D. melanogaster*, TH-20 specifically inhibits in vitro JHB₃ biosynthesis in the Medfly by specifically inhibiting epoxidation of the unique C_(6,7) double bond in a dose-dependent manner, but does not inhibit epoxidation at the C_(10,11) double bond (Figure 9). Therefore, JH-III levels are not affected in a dose-dependent manner. No inhibition of FR-7 is evident in the presence of 10⁻⁷M to 10⁻⁵M of TH-20, and some increase is observed at the higher concentration of 10⁻⁴M (Figure 9).

Figure 9: Dose-response of virgin female *D. melanogaster* CA to TH-20 as expressed by in vitro production of JH homologs

Finally, the effect of TH-20 on oviposition by virgin *C. capitata* was examined. This inhibitor does not reduce the level of oviposition when 1 microliter aliquots of a 10^{-4} M solution in acetone (experimental) or 1 microliter acetone (control) were applied on D-1 after eclosion and oviposition monitored for the following four days. In the control group, 27% of the females deposited an average of 32 eggs over the four-day period. In the experimental group, 33% deposited an average of 26 eggs per female.

Cloning putative JH-related genes from C. capitata

We attempted to clone a homologue of the *ap* gene of *Drosophila* from the Medfly, using the PCR technique. We believe that the *ap* gene in the Medfly should play a similar role to the one played by *ap* in *Drosophila*. Therefore, it is conceivable that domains that are important for the function of the gene will be conserved in the Medfly. These domains include the LIM domains and the Homeo-domain.

Degenerate PCR primers derived from the regions thought to be conserved, were designed using the codon usage of Medfly. Their sequence and location on the sequence of the *Drosophila ap* cDNA sequence are shown in Figure 10. PCR reactions were performed using genomic DNA from the Medfly and were carried out at several annealing temperatures (50-58°C) implying more or less stringent conditions. PCR products (Figure 11) were transferred to a membrane and hybridized with a probe prepared from the *ap* cDNA from *Drosophila* (Figure 12). A 570 bp fragment hybridized to the probe. This fragment was cloned in pGEM (Promega) and sequenced.

As can be seen in Figure 13, the first 464 bp of the cloned fragment are 98% identical to those found in the corresponding region in the *Drosophila ap* cDNA including the 3' LIM and Homeo-domain. The remaining 100 bp of the fragment are not homologous to the *Drosophila* gene.

To further confirm that the origin of the PCR product is indeed the Medfly genome, we used it as a probe in a Southern blot analysis performed on digested genomic DNA of Medfly and *Drosophila* (as a positive control) (Figure 14). This Southern blot was performed under stringent conditions (at 65°C) since the probe is supposed to be 100% homologous to the Medfly genome. Both *Drosophila* and Medfly DNA hybridized to the probe although different hybridization patterns were

obtained from the two species. These results indicate that the fragment that we cloned and sequenced originates in the Medfly.

Taken together, these results indicate that a homologue of the *ap* gene of *Drosophila* exists in the Medfly genome. In order to clone the whole gene, we used a cDNA library (prepared in gamma-ZAPII, Stratagene) from third instar Medfly larvae using the PCR amplified fragment as a probe. From studies performed in *Drosophila*, we know that the *ap* transcript is abundant at this developmental stage. We therefore expected that this transcript would appear at this stage also in the Medfly.

Unfortunately, no positive phages were obtained from the screening of this cDNA library. Therefore, a gamma-EMBL4-N genomic library of the Medfly was probed with the PCR amplified fragment as a probe. Five positive phage clones were thus isolated. Following secondary and tertiary screening only one of the five clones proved to be positive. DNA from the positive phage was extracted, subjected to restriction mapping and Southern analysis. This allowed the identification of a 4.5 kb fragment from this phage that hybridized to the probe. This fragment was subcloned in the pBluescript vector and was sequenced. However, the resulting sequence proved to have no homology to the probe or to *Drosophila ap* gene. We suspect that its hybridization to the probe was an artifact.

Discussion

From previous studies on other insects, primarily *D. melanogaster*, mating was expected to increase JH levels and accelerate allatal maturation. In fact, on comparing the time course and levels of total JH homologs produced by virgin and mated Medfly females, the increase in synthesis of JH in vitro was found to be retarded by one day in females that have mated, and peak production was somewhat reduced (Figure 1).

Postmating control of hormonal activities associated directly or indirectly with reproduction may be regulated endogenously by cerebral factors or may be attributed to exogenous, male derived-seminal fluid components. These alternatives are not mutually exclusive. Cerebral regulation of CA maturation by allatostatic neuropeptides is common, and may partly explain the observed reduction in JH production that mating has on total JH production in the Medfly (Figure 2).

In order to explain this apparent reduction in JH biosynthesis one possibility that comes to mind might be that mating, and its subsequent effects on cerebral

regulation of allatal maturation, might lead to differential synthesis of specific JH homologs. HPLC and TLC separations of JH homologs from incubation media of virgin Medfly CA demonstrated the anticipated presence of JHB₃, with JH-III as a minor component, and revealed a third component, FR-7 (Figure 3). This raised several issues, one of them being its chemical identity. This was pursued in collaboration with Professor R.R.K. Rickards of the Australian National University in Canberra. Much effort was invested in incubating and pooling thousands of Medfly CA preparations and isolating the FR-7 peak by HPLC, in order to perform mass spectrometry. The structure of FR-7 is not yet resolved.

The second concern was whether this peak was of physiological importance in Medfly reproduction, or not. Several results are suggestive. Firstly, levels of FR-7 were **not** significantly reduced when CA are incubated with axonal connections to the brain intact (Figure 4). This is consistent with enhanced oviposition in mated females. FR-7 is a more persistent product of the Medfly CA: When a time-course of incubations, in increments of 2 h and up to 8 h, was performed, levels of FR-7 increased consistently even after JHB₃ biosynthesis had leveled off (Figure 6). Thus, FR-7 is an endogenous product of the Medfly CA.

Mating in many insects is associated with the transfer of accessory gland peptides in the seminal fluid, some with stimulatory effect on JH production, as we have previously shown with *D. melanogaster* and *H. armigera* CA (Moshitzky et al., 1996; Fan et al., 1999). As mentioned above, mating stimulates oviposition. In addition to the indirect affect via allatostatic control of maturation, which is a developmental process, we also examined the effects of the heterologous *D. melanogaster* SP on JH homolog production by Medfly CA in vitro. The result was that JHB₃ biosynthesis was **reduced**, whereas FR-7 synthesis was significantly stimulated (Figure 7). This too is consistent with a possible role of FR-7 in oogenesis, as conventional thought is that JH is directly involved in the stimulation of yolk protein biosynthesis and its uptake by the developing oocyte. However, the observation that SP stimulates FR-7 production is not direct evidence of its positive role in Medfly reproduction. This particular result does suggest that JHB₃ may not be significant for oogenesis, a conclusion which, if correct, impinges on the possibility of controlling Medfly reproduction by specifically inhibiting the production of this unique dipteran JH.

Two candidate inhibitors of P450 monooxygenases, KK-42 and TH-20, were assayed for their effect on JH precursor epoxidation in *D. melanogaster* and *C. capitata*. The first, KK-42, has been proposed as an inhibitor of ecdysteroid synthesis in insects (Yamashita et al., 1987). Both inhibit epoxidation at $C_{(10,11)}$ and $C_{(6,7)}$ (Figure 8). In the *Drosophila* system, KK-42 is a more specific inhibitor of epoxidation of the $C_{(6,7)}$ double bond, which is essential for production of JHB₃ in the higher Diptera. The slight increase in JH-III in the presence of both inhibitors indicates that when the epoxidation at $C_{(6,7)}$ is essentially blocked, some of the 10,11-epoxy FA produced from precursor FA is methylated to JH-III, while at the same time, the appreciable increase in MF at high concentrations of TH-20 indicates that precursor FA itself is methylated to MF in the absence of epoxidation at $C_{(10,11)}$. KK-42 has no detrimental effect on oviposition in either virgin or mated *D. melanogaster*, although control levels of oviposition are appreciably higher in mated females (Table 1). On the other hand, TH-20 is a specific inhibitor of the $C_{(6,7)}$ double bond in the Medfly, but has no apparent effect on the $C_{(10,11)}$ double bond. FR-7 production too is not depressed, and if anything, is even stimulated at the highest inhibitor concentration of 10^{-4} M (Figure 9). This suggests that FR-7 does not contain a $C_{(6,7)}$ epoxide function. It appears that neither ecdysteroids (the product initially described as inhibited by KK-42; see Yamashita et al., 1987) nor JHB₃ biosynthesis, are essential for egg development and deposition in both *D. melanogaster* and *C. capitata*. TH-20 has no detrimental effect on the percentage of Medfly females depositing eggs, nor on average oviposition over 4 days. This supports the possibility that FR-7 may be involved in Medfly fecundity. The above analysis is based on correlations that may not be causative. Direct evidence that FR-7 is indeed of physiological significance in the control of Medfly reproduction requires demonstration of such significance by the assay of synthetic FR-7 at physiological doses. In the absence of a definitive chemical structure for FR-7, such assays are precluded at this time.

From this present study, we conclude that an *apterous* homolog exists in the Medfly genome but despite our efforts, we were unsuccessful in isolating it with a cDNA library from third instar Medfly larvae. We anticipate that screening of better genomic libraries and cDNA libraries from other developmental stages should allow isolation of the Medfly *ap* homolog.

Scientific implications

The major implication is that significant differences occur in the array of JH homologs and related compounds that are produced by the CA of different higher Diptera. JHB₃ seems to be a common feature, with varying amounts of JH-III produced, and under certain circumstances, methyl farnesoate. In the case of the Medfly, the object of this present study, an additional related compound incorporating a methyl moiety from donor methionine, is produced endogenously. Specific JH homologs are commonly regarded as major gonadotropins in insects. In the case of *D. melanogaster*, the very low levels of JH-III initially identified were disturbing, but subsequent identification of appreciable amounts of JHB₃, a unique product of dipteran CA, seemed to resolve this issue. The results of this present study now suggest that inhibition of JHB₃ biosynthesis (and presumably ecdysteroid biosynthesis as well) by substituted imidazole inhibitors of monooxygenases, does not interfere in the normal course of egg production in both *D. melanogaster* and *C. capitata*. Concurrent biosynthesis of FR-7 in the Medfly is not depressed by cerebral allatostatic control of JH biosynthesis by the adult CA, and increases moderately when JH homolog production is inhibited by the epoxidase inhibitors. What this means in terms of reproductive regulation in this and additional flies is worthy of further attention.

A presumptive *apterous* gene exists in the Medfly, but until localized in the Medfly genome and mutants become available for possible rescue of reproduction by FR-7, no conclusions can be drawn of the relative significance of FR-7 or JH homologs in reproductive control.

Potential impact

It is too premature and conjectural to evaluate whether this study will serve for developing a procedure for reproductive control of the Medfly, and any evaluation of environmental impact is dependant possible agricultural impact.

1 GACGGCTGGTGTCTCAGATTCTAAGTAGATCAGCTGCAGACCAAGTCCGGTAGTCGAGTTTCGAATTTAAGTCGGTTTGTGTTTGGAAAGCGGTGCGCGTATTGCGATTTTGTGTTT
121 GACTTGAGCTCGGCTGTAGTTTCGGTTTGGCAGAATCGATCGAAGGAGAATCGACACAGAAACAGGGAGTTGGCGAAACGGATACAAAACCTGAAGTTGGATCGGTAATCGCGTGCCTGG
241 TCATAAACACAAACAAGGGAGCACAGCCGCATACGCATACGGCGCTCTGTCTCTAATGCCTTATCAGTAACAGGCATCGTCGAGAGTCGTAGGAAGAGCCGACAAAGGCCGACCTTT
cAP-B
361 ACCACTCAATTTGGATCTGATGTGCCATTCTCGCGCTGTCTCGTAAATTAATGTCTATAAATTAACTTTAGATTTCTTACGCTAACCGGTGGTGTGTCAGCGGTCCGAAAAACGCTTTTGGCG
481 TCAGATACAGGGGCGGTCCCTAATCAATACAAATATCTTACCTGCCGCGAGTCAAGTGTGTTTGTAGTGTGTGACCTATGTTCTTATATACCTTGGTGCTAAGGAGAAAGTTTA
601 AAAAAAGCGGCATTCAATCTGATGTTTGTGCAATAAGAAAAGGCAAGCCATGGTGTCAATCTCTAAGACAACGCCGAAGTGATGCATGTCCGCAAAGTAATAGATCAAGTTGTGCGCAC
XbaI
721 ACTTATTTCCGGCGAGCCTAGCCGGGCATCTAATCGTGGCCAGTGTCAAGTGTCCAATAGCACATCTAGCCGGGTCTAGAGGATTACCCAAGACCAACGTAGCACCGCAGCCCTTTTAA
841 GGAGGCATAAGGCTCGCAAGACACAACGCACAGCCTACACAACGACAAACATAACACGACCGGACATCATCTTGGAAATAGACCTCGTGTCTTCCAAAAGACGCAC ATG GGC
1 Met Gly
958 GTC TGC ACC GAG GAG CGC CCT GTG ATG CAT TGG CAG CAG AGC GCA AGA TTT CTT GGG CCC GGC GCA AGG GAG AAA AGT CCA ACA CCA CCT
3 Val Cys Thr Glu Glu Arg Pro Val Met His Trp Gln Gln Ser Ala Arg Phe Leu Gly Pro Gly Ala Arg Glu Lys Ser Pro Thr Pro Pro
PstI
1048 GTA GCA CAT CAA GGG AGC AAT CAA TGT GGC AGT GGT GCA GGT GCA AAT AAC AAT CAC CCA TTG TTC CGC GCG TGC TCC TCA TCC TCG TGT
33 Val Ala His Gln Gly Ser Asn Gln Cys Gly Ser Ala Ala Gly Ala Asn Asn Asn His Pro Leu Phe Arg Ala Cys Ser Ser Ser Ser Cys
1138 CCA GAT ATT TGT GAT CAT AGT ACA AAG CCA TTT GGC AAC GCT TAC GGC ACC GAG TCA TTT AGA AGC TAC GAA ACA GCC GAT CGT GCT ACC
63 Pro Asp Ile Cys Asp His Ser Thr Lys Pro Phe Gly Asn Ala Tyr Gly Thr Glu Ser Phe Arg Ser Tyr Glu Thr Ala Asp Arg Ala Thr
1228 TTT GAG GAC TCA GCC GCC AAA TTC TCC ATC AGC CGC AGT CGA ACA GAC TGC ACG GAG GTC ACG GAC GAG ACG ACG TCG GGC ATA TCA TTC
93 Phe Glu Asp Ser Ala Ala Lys Phe Ser Ile Ser Arg Ser Arg Thr Asp Cys Thr Glu Val Ser Asp Glu Thr Thr Ser Gly Ile Ser Phe
LIM 1
1318 AAG ACC GAA CCC TTC GGA CGC CCC AGC AGT CCC GAG TCC ACA AGC GAT AGC AAA ATA ACG CGC AAC CTC GAC GAC TGC TCC GGC TGC GGA
123 Lys Thr Glu Pro Phe Gly Pro Pro Ser Ser Pro Glu Ser Thr Ser Asp Ser Lys Ile Thr Arg Asn Leu Asp Asp Cys Ser Gly Cys Gly
108 CGT CAG ATA CAG GAT CGC TTC TAC CTC TCC GCT GTG GAA AAA CGG TGG CAT GCA AGT TGC CTA CAG TGC TAC GCC TGT CGG CAG CGC CTG
53 Arg Gln Ile Gln Asp Arg Phe Tyr Leu Ser Ala Val Glu Lys Arg Trp His Ala Ser Cys Leu Gln Cys Tyr Ala Cys Arg Gln Pro Leu
LIM 2
1498 GAA CGG GAA TCC TCA TGC TAC TCA CGT GAC GGC AAC ATT TAT TGC AAA AAC GAT TAT TAT AGT TTT TTT GGT ACT CGC CGA TGC TCG CGC
183 Glu Arg Glu Ser Ser Cys Tyr Ser Arg Asp Gly Asn Ile Tyr Cys Lys Asn Asp Tyr Tyr Ser Phe Phe Gly Thr Arg Arg Cys Ser Arg
BsaHI
1588 TGC CTG GCC TCC ATC AGC TCC AAC GAG CTG GTC ATG GGC GGC AGA AAT CTT GTT TTT CAC GTC AAC TGC TTC TGC TGC ACT GTC TGC CAC
213 Cys Leu Ala Ser Ile Ser Ser Asn Glu Leu Val Met Arg Ala Arg Asn Leu Val Phe His Val Asn Cys Phe Cys Cys Thr Val Cys His
PstI
1678 ACG CCA CTG ACA AAG GGA GAC CAG TAC GGC ATC ATC GAC GGC CTC ATC TAC TGC AGC ACC CAC TAC AGC ATA GCC AGG GAG GGC GAT ACC
243 Thr Pro Leu Thr Lys Gly Asp Gln Tyr Gly Ile Ile Asp Ala Leu Ile Tyr Cys Arg Thr His Tyr Ser Ile Ala Arg Glu Gly Asp Thr
XhoI
1768 GCC TCA TCC AGT ATG AGC GCC ACC TAC CCG TAC AGC GCC CAG TTC GGC TCA CCC CAC AAC GAC TCG TCG AGC CGC CAC TCG GAC CCT AGT
273 Ala Ser Ser Ser Met Ser Ala Thr Tyr Pro Tyr Ser Ala Gln Phe Gly Ser Pro His Asn Asp Ser Ser Pro His Ser Asp Pro Ser
1858 CGG AGC ATT GTT CCT ACG GGC ATC TTT GTG CCC GCG TCC CAC GTA ATC AAC GGA CTG CGC CAG CCA GCT CGT CAA AAG GGC AGG CCC CGC
303 Arg Ser Ile Val Pro Thr Gly Ile Phe Val Pro Ala Ser His Val Ile Asn Gly Leu Pro Gln Pro Ala Arg Gln Lys Gly Arg Pro Arg
BglII
1948 AAG CGC AAG CCC AAG GAC ATC GAG GCG TTC ACC GCT AAC ATA GAT CTC AAC ACT GAG TAC GTG GAC TTT GGC CGA GGC TCG CAC CTA AGC
333 Lys Arg Lys Pro Lys Asp Ile Glu Ala Phe Thr Ala Asn Ile Asp Leu Asn Thr Glu Tyr Val Asp Phe Gly Arg Gly Ser His Leu Ser
homeodomain
2038 TCC TCG TCG GGC AGC AAA CGA ATG CGA AGC TCG TTT AAG CAT CAC CAG CTG CGT AGC ATG AAG TCC TAC TTT GGC ATT AAT CAT AAC CCT
363 Ser Ser Ser Arg Thr Lys Arg Met Arg Thr Ser Phe Lys His His Gln Leu Arg Thr Met Lys Ser Tyr Phe Ala Ile Asn His Asn Pro
2178 GAT GCA AAG GAT CTA AAA CAA TTG TCA CAG AAA ACT GGT TTA CCA AAG AGG GTC CTA CAG GTC TGG TTT CAA AAT CCA AGG GGC AAA TGG
393 Asp Ala Lys Asp Leu Lys Gln Leu Ser Gln Lys Thr Gly Leu Pro Lys Arg Val Leu Gln Val Tyr Phe Gln Asn Ala Arg Ala Lys Trp
2218 GCG GCG ATG ATG ATG AAG CAG GAT GGC AGC GGC CTG CTG GAG AAG GGC GAA GGC GCT CTG GAC CTC GAC AGC ATC TCC GTG CAC AGT CCT
423 Arg Arg Met Met Met Lys Gln Asp Gly Ser Gly Leu Leu Glu Lys Gly Glu Gly Ala Leu Asp Leu Asp Ser Ile Ser Val His Ser Pro
2308 ACG TCG TTT ATA TTG AGA GGA CCG AAC AGC ACA CCG CCA CTT AAC TTG GAC TAA CCGATGCTCAGGAAACGCCGAAACAAAATGATAGTGTGACAAAGGAAATG
453 Thr Ser Phe Ile Leu Arg Gly Pro Asn Ser Thr Pro Pro Leu Asn Leu Asp ***
XbaI
410 GGCGCAGCAGTCACTCCGACATCATACGTTCTTATGCCAGTTGTCTATTGCGGTGCCTCATACACAGTCCCGGACCCGGCTTGACTACTGAATCCGGATACCTGTCTAGATCAAGCAGG
SmaI
2530 GCTAGCTCTTGAATGCTATGGGCTGCCTTGTGGATGGGAATCAATTGGAAAGCTTGTCTTGTGTTTCAAAATCGAAAAATTGATTCATCCACAGGTTAAACGCCCGGAAACGCTCGG
2650 AAAATGCTAAGAAAATATGTATTTTGTATTTGGCTATTGACCGTTTCCAGCACTACTCGCCTCGTCTTGGCCACATGTTGTTTCTATAGTTTAAACGTTCACTTGTAAATATGTACT
2770 GTAATCACATAGTTTATTTCAAACGCATCCATATTAGCCTGACTCCCTGTTTGTGATTTCTTTAAAGCGCAGAATATCAATTCGAACATTGAAATATGAATCAAAAGAAGGTGGAT
2890 TCATGCAAAGATTACCAACTGTAAAGTTCTTTCTAAAACTGTCATAATAATTAAGAAAAACAACTTCGTTAAATTTCAATTTGTATAGAAAATAGTTATGTTACTATACCAGTCAAAA
3010 TTTTAAACATCAACATGCAGAATTTACGAATTAAGTATGACTAAAACTTTTAAATACCAACAGCGAAACGTGTGATATTTAAATTTGTTTTTCTTTTTCAGTGACACGACTA
3130 AAAGATACCCGTTACATTGTAAATAATCTTGCAATATAGATGCGGCGAGGTCAATGCCCTTCCCTCATATGAATTGCCCGCTGGCAATGTTTTACCAAGCTTTCACCTTCCGATTAGGT
3250 TTGGTGTCTATTAAAGTAAATCCGATTAAACTTTGTTTTTAATACGGGAGCGGTGTCACCTTCCTTTCCGGTATAAAAACATAGGTGAGGAAAAAGTTTGAGGCCAACCTTACGGCGCACCA
3370 AGCGGCACACAAATGAAATGAAATCTTTAGCTTCTATAGTAGTCGAAAAACGATCCTTATCAAGCATAAATGCTTATATCTCTGTAAACATTCTTTTGACCACTAGTTTACACGTA
3490 TGTTCTACGAGGAACGACCGATTGTCTATGAGAGCTATAGGTTTACCATATGATATGCATGCATTACTTCAGCTCCATCTCTTTTGTATTCATAAACGTATAATGGTTTGAGAGTA
3610 AACATTTTGTGGCTATATAGTTCAAGTTTACATATTAGAGTACCGTGCCCAAGACGCGCTTTAAAAATATTAAGCGGTTGTAAATGAAGTCCCATATGTATAATGTCTAGGTACA
3730 TGGATATTGTTAATATATTATTATTTTATTTAAAGAGCTCTCAACAATATGTATGAAGAAATCTGCGTATATCAACATATAATGAAATGCTGATGCATTCTGATTATATATATGTAAT
3850 AATATTTATTGCCAAATTTAATAGTTAGAGACGAATTTAGTTTGAACAGACTATACATAAAGTCCATATGTACATGTAGTATTACGGTAAAGTAATAGCTTCGCTCAAGCATACGAC
3970 CAGGTTCTGAAGCTATTAATACGAACTGTAATTATAATCAATCAGTAAACACTGGAAGATTGTACTTACAAATAGTTAACTCATAAGTCTTACCATAGAAGCTTAAATAAACGTAT
4090 TCAAGCAAA

Figure 10. The sequence of the *Drosophila ap* cDNA is shown. The locations and directions of the primers used for PCR reactions are shown in red arrows. The LIM1 and LIM2 domains are shown in pink, and as well as the homeodomain is marked in yellow.

Figure 11: PCR products of reactions in which the primers shown in Figure 1 were used. Lane 1 (on the left): control reaction in which the *Drosophila ap* cDNA was used as a template, performed at an annealing temperature of 58°C. Lanes 2-6: reactions performed on genomic DNA extracted from the Medfly as a template, at annealing temperatures from 50°C to 58°C.

Figure 12: Hybridization of PCR products (seen in Figure 2) with the *Drosophila ap* cDNA as a probe. Lane 1 (on the left): control reaction with the amplified fragment from the *Drosophila ap* cDNA. Lanes 2-6: amplified fragments of Medfly genomic DNA from PCR reactions performed at annealing temperatures from 50°C to 58°C.

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1  ACTGCCGTACCCATTACAGCATAGCCAGGGGAGGGGGATACCGCCTCATC  50
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
1727 ACTGCAGGACCCACTACAGCATAGCCA.GGGAGGGGGATACCGCCTCATC  1775

51  CAGTATGAGCGCCACCTACCCGTACAGCGCCAGTTCGGCTCACCCACCA  100
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
1776 CAGTATGAGCGCCACCTACCCGTACAGCGCCAGTTCGGCTCACCCACCA  1825

101  ACGACTCCTCGAGCCCGACCCGGACCTAGTCGGAGCATTGTTCTCTACG  150
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
1826 ACGACTCCTCGAGCCCGACTCGGACCTAGTCGGAGCATTGTTCTCTACG  1875

151  GGCATCTTTGTGCCCGCGTCCACGTAATCAACGGACTGCCGCGCCAGC  200
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
1876 GGCATCTTTGTGCCCGCGTCCACGTAATCAACGGACTGCCGCGCCAGC  1925

201  TCGTCAAAAGGGCAGGCCCGCAGCGCAGCCCAAGGACATCGAGGCGT  250
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
1926 TCGTCAAAAGGGCAGGCCCGCAGCGCAGCCCAAGGACATCGAGGCGT  1975

251  TCACCGCTAACATAGATCTCAACACTGAGTACGTGGACTTTGGCCGAGGC  300
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
1976 TCACCGCTAACATAGATCTCAACACTGAGTACGTGGACTTTGGCCGAGGC  2025

301  TCGCACCTAGCTCCTCGTCGCGCACCAACGARTGCGACCTCGTTTAA  350
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
2026 TCGCACCTAGCTCCTCGTCGCGCACCAACGARTGCGACCTCGTTTAA  2075

351  GCATCACCAGCTGCGTACCATGAGTCCTACTTTGCCATTATCATAACC  400
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
2076 GCATCACCAGCTGCGTACCATGAGTCCTACTTTGCCATTATCATAACC  2125

401  CTGATGCAAGGATCTAAACAATTGTCACAGAAACTGGTTTACCAAG  450
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
2126 CTGATGCAAGGATCTAAACAATTGTCACAGAAACTGGTTTACCAAG  2175

451  AAGGTCCTACAGGT  464
   |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
2176 AAGGTCCTACAGGT  2189

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Figure 13 An alignment between part of the *Drosophila ap* cDNA (lower sequence) and part of the cloned Medfly sequence (upper sequence) is shown. The first 464 bases cloned from the Medfly are 98% identical to part of the *Drosophila ap* cDNA, including the 3' LIM domain and the Homeodomain. The remaining 100 bp of the cloned sequence, which are not homologous to the *Drosophila ap* cDNA, are not shown

Figure 14 Southern blot analysis of *Drosophila* and Medfly genomic DNA. DNAs were digested with *EcoRI* and the membrane was probed, under stringent conditions, with the amplified PCR fragment derived from Medfly (see text).

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