table. Both 3-MC and PB treatment significantly decreased the K_{m2} with respect to the control microsomes.

The highest activity found for the dinemorphan N-demethylase was about one third that of either aminopyrine N-demethylase[16] or ethylmorphine N-demethylase[17] activities determined with microsomes from uninduced mice of the same strain. Phenobarbital treatment increased both the V_{max1} and V_{max2} 5 times per mg of microsomal protein and more than 2 times per nmol of cytochrome P-450, compared to microsomes from control mice.

Thus it appears that PB specifically induced the cytochrome P-450 species which catalyzes the N-demethylation of dinemorphan. Such specificity, in mice of the same strain, was not found for aminopyrine N-demethylase (our unpublished results) or ethylmorphine N-demethylase[17] activities although similar extents of PB-induction, shown by an increase in microsomal protein, were reported. In rats, however, benzphetamine N-demethylase activity was found to be specifically induced by PB treatment[18].

3-MC-treatment did not influence the V_{max} with respect to the control if related to mg of protein, but decreased V_{max1} and V_{max2} if related to nmoles of cytochrome P-450 (table). This shows that 3-MC-inducible cytochrome P-450 species seem to be completely unable to demethylate dinemorphan.

Moreover, dinemorphan was able to bind to the active site of cytochrome P-450, giving a type I binding spectrum. A greater ΔA_{max} was found with PB-microsomes than with 3-MC-microsomes (data not shown).

Dinemorphan demethylation was inhibited by CO and other classical monooxygenase inhibitors such as SKF-525A and metyrapone[19]. SKF-525A strongly inhibited dinemorphan N-demethylase activity in microsomes from PB-induced mice (fig. 3A), while metyrapone, a stronger inhibitor than SKF-525A at all concentrations investigated, is more active towards microsomes from 3-MC-induced mice (fig. 3B).

In conclusion, these results show that dinemorphan, a morphinan-type antitussive drug similar to dextrometorphan, is N-demethylated by liver microsomal monooxygenase and specifically by that from PB-treated mice. Thus, the dinemorphan demethylation can potentially be used as a 'diagnostic' substrate to show if any xenobiotic or treatment, given to mice, is able to induce the PB-like form of cytochrome P-450.

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Migration and dispersal of spermatozoa in spermathecae of queen honeybees (*Apis mellifera* L.)

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Summary. Observations of variability in the phenotypic composition of honeybee colonies through time have led to ad hoc hypotheses concerning the distribution of spermatozoa in multiply mated honeybee queens. These hypotheses are not supported by available data. Serial sections of spermathecae from honeybee queens were examined by light microscopy. No obvious physical evidence was observed of agglomerations, aggregations, clumps, layers, or wads of spermatozoa within spermathecae within 24 h of insemination. A hypothesis is proposed explaining the occurrence of fluctuations of progeny phenotypes.

Taber[1] showed by progeny analysis that sperm utilization by multiply inseminated honeybee queens is not random. He concluded from his study that honeybee spermatozoa are not mixing appreciably within the spermathecae of queens and that spermatozoa are possibly clumping together. However, a look at his data shows that both distinguishable progeny phenotypes were present in significant proportions for all samples of all queens analyzed[2,3].

Kerr et al.[4] proposed high genetic relatedness among honeybee nestmates as a consequence of the physical 'aggregation' and 'agglomeration' of spermatozoa in the spermathecae of queens. Their data, like those of Taber[1],

show the presence of both distinguishable phenotypes in almost all samples for all queens. Two important characteristics of their data are that phenotypic frequencies tend to fluctuate around 50%, and the fluctuations clearly decrease over time.

Page and Metcalf[3] investigated sperm utilization of 1-year-old honeybee queens by allozyme analysis. Their data show fluctuations in genotypic frequencies, again suggesting non-random sperm utilization. However, they show that queens use the sperm of at least 3 males at any time and are probably using the sperm of at least 5 or 6. Laidlaw and Page (unpublished data) have subsequently demonstrated that queens instrumentally inseminated with semen from 6 males use the spermatozoa of all 6 males at all times in significant proportions, supporting the hypothesis that the spermatozoa are mixing appreciably in the spermathecae of queens.

The purpose of the present study is to examine by histological serial sectioning, and microscopic examination, the spermathecal contents of instrumentally inseminated honeybee queens during the time of active sperm migration from the oviducts to the spermatheca in an attempt to better understand the process of sperm migration and mixing of spermathecal contents.

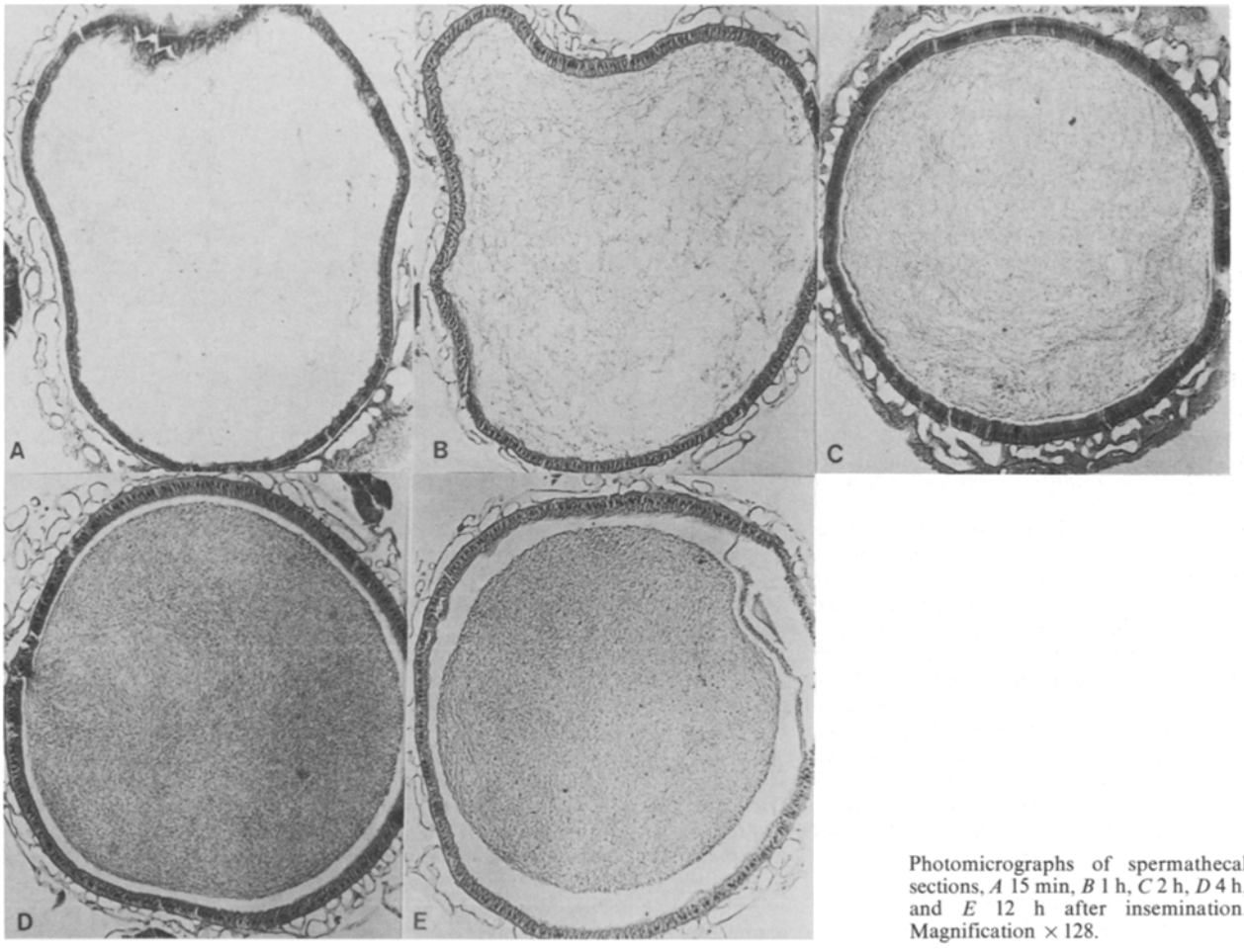
Materials and methods. 24 virgin queens were obtained from the Bee Biology Facility, University of California, Davis. Instrumental insemination of queens was by the method of Laidlaw[5]. Each queen was inseminated with 2 successive 4 µl aliquots of semen from 3–5 different drones, arranged in serial order in the syringe. This simulated successive matings by the queen.

After insemination, queens were maintained in the dark at 27 °C and 40% relative humidity. At intervals of 5, 10, 15, 30 min, and 1, 2, 4, 8, 12, and 24 h, 1 or 2 queens were quickly dissected (only taking a few sec), their spermathecae removed, and rapidly fixed in buffered 4% (pH 7.2) glutaraldehyde at 4 °C. This rapid fixation process was designed to minimally disturb the physical distribution of spermatozoa, although we cannot be certain we did not alter it somewhat.

Standard histological techniques were used to section the spermathecae. Spermathecae were dehydrated using a butyl alcohol series, embedded in Paraplast™ and sectioned at 10 µm. Sections were dewaxed, hydrated, and stained with Giemsa at pH 4.6 for 30 min then examined by light microscopy.

The use of labeled spermatozoa may have improved the resolution of this experiment, however, they must either be incubated in solutions of radioactive isotopes or fluorescent dyes, or labeled isotopes must be fed to the drones and incorporated into the spermatozoa. The first method may alter the migration and dispersion behavior of the spermatozoa. Since drones can not be laboratory reared, radioactive compounds must be fed to free-flying experimental colonies. This introduces the undesirable probability of spreading the labeled material throughout the experimental apiary by intercolony drifting of workers and robbing behavior.

Results and discussion. No agglomerations, aggregations, clumps, layers, or wads were visually detected in any of the sectioned spermathecae (see fig.). Spermatozoa apparently migrate into the spermatheca and disperse throughout the



Photomicrographs of spermathecal sections, *A* 15 min, *B* 1 h, *C* 2 h, *D* 4 h, and *E* 12 h after insemination. Magnification × 128.

available space, with the spermatheca becoming more densely packed with a longer time since insemination.

The most parsimonius explanation for the reported fluctuations of phenotypic frequencies, lies in the process of sperm migration and the filling of the spermatheca. Queens mate with many drones in rapid succession and receive the semen of these drones into the distensible lateral oviducts[6]. Spermatozoa migrate into the spermatheca by active and passive mechanisms over a period of about 24 h[7-9]. It is likely that spermatozoa deposited at the anterior ends of the lateral oviducts (the greatest distance from the spermathecal duct) are last to reach the spermatheca. As the spermatheca becomes more densely packed, there is less room for spermatozoa to distribute themselves and, therefore, a higher probability that like ('brother') spermatozoa will be distributed in close proximity to each other. As they become more densely packed, they may tend to become oriented parallel to each other through random movement in a confined space, thus forming the characteristic 'whorls' of sperm shown by others[10,11].

It is unlikely that instrumental insemination causes anomalous results. Taber[1] showed similar phenotypic fluctuations in both naturally mated and instrumentally inseminated queens. Furthermore, both Bishop[12] and Laidlaw[6] have shown that queens returning from mating flights have very few (if any) spermatozoa in their spermathecae, demonstrating that the male ejaculation itself does not directly fill the spermatheca.

The queen may be actively mixing some of the spermatozoa in her oviducts by contracting the abdomen and the thin muscular layer that covers the oviducts[6]. The adaptive significance of mixing these sperms would be to increase the effective number of matings[13].

Decreasing fluctuations in observed phenotypes can be explained by the sperm utilization model of Harbo[14]. Harbo presented data that suggest honeybee queens release a constant volume of spermathecal fluid each time they fertilize an egg. The spermathecal fluid is then replaced. This leads to a rapid decrease in the density of spermatozoa in the spermatheca and, hence, more potential for mixing. A rapid decrease in fluctuations is expected and is demonstrated by the data of Kerr et al.[4].

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Quercetin interacts with calmodulin, a calcium regulatory protein[1]

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Summary. Quercetin was found to interact with the Ca^{2+}-calmodulin complex, suggesting that it may act as a calmodulin antagonist in vivo to inhibit calmodulin-dependent phenomena caused by biologically active agents, including tumor promoters.

It has been reported that quercetin, a flavonoid, has mutagenic activity[2-6], although it was found to be non-carcinogenic in animal tests[7-9]. We have recently found that quercetin shows inhibitory effects on a wide variety of tumor promoter-induced phenomena, and suggested that the anti-tumor-promoter activity of quercetin may result in lack of development of tumors, despite its mutagenicity. Calmodulin is known to be the major calcium-regulatory protein in various kinds of cells, and is reported to participate in the mechanism of action of tumor promoters[12-16]. Therefore, it appeared interesting to investigate the effect of quercetin on the Ca^{2+}-calmodulin system to know why quercetin shows anti-tumor-promoter activity. In this communication, we present evidence suggesting that quercetin acts as a calmodulin antagonist; this characteristic may be involved in the mechanism of the anti-tumor-promoter action of quercetin.

It has been reported that fluorescence of N-phenyl-1-naphthylamine (NPN) is enhanced in the presence of Ca^{2+}-calmodulin complex, and that calmodulin antagonists depress the enhanced fluorescence[17,18]. In order to demonstrate the interaction of quercetin with calmodulin, we examined the effect of quercetin on the enhanced fluorescence of NPN induced by binding with calmodulin. Calmodulin was obtained from Boehringer Mannheim Yamanouchi Co., Tokyo. N-Phenyl-1-naphthylamine was purchased from Wako Pure Chemical Industries, Ltd., Osaka. Fluorescence measurements were carried out at room temperature using a Shimadzu fluorescence spectrophotometer (Model RF-502). As shown in figure 1, enhanced fluorescence of NPN induced by the addition of calmodulin was completely depressed by quercetin at the concentration of 50 μM, and it was suggested that quercetin interacted with calmodulin.

In order to confirm this possibility, we tried to show the direct interaction of quercetin with calmodulin; that is, the binding activity of ^{14}C-quercetin to Ca^{2+}-calmodulin complex was examined by the equilibrium binding technique of