Hiding messages in DNA microdots

The microdot is a means of concealing messages (steganography)[1] that was developed by Professor Zapp and used by German spies in the Second World War to transmit secret information[2]. A microdot ("the enemy's masterpiece of espionage"[2]) was a greatly reduced photograph of a typewritten page that was pasted over a full stop in an innocuous letter[2]. We have taken the microdot a step further and developed a DNA-based, doubly steganographic technique for sending secret messages. A DNA-encoded message is first camouflaged within the enormous complexity of human genomic DNA and then further concealed by confining this sample to a microdot.

A prototypical 'secret message' DNA strand contains an encoded message flanked by polymerase chain reaction (PCR) primer sequences (Fig. 1a). Encryption is not of primary importance in steganography, so we can use a simple substitution cipher[1] to encode characters in DNA triplets (Fig. 1b). Because the human genome contains about 3×10^9 nucleotide pairs, fragmented and denatured human DNA provides a very complex background for concealing secret-message DNA. For example, a secret message 100 nucleotides long added to treated human DNA at one copy per haploid genome would be hidden in a roughly three-million-fold excess of physically similar DNA strands. Confining such a sample to a microdot might then allow even the medium containing the message to be concealed from an adversary. However, the intended recipient, knowing both the secret-message DNA PCR primer sequences and the encryption key, could readily amplify the DNA and then read and decode the message.

Even if an adversary somehow detected such a microdot, it would still prove extremely difficult to read the message without knowing the specific primer sequences. For example, if 20-base random primers were used to amplify the DNA, separate amplifications with more than 10^{20} different primer pairs would be required, even allowing three mismatches per primer, followed by analysis of any PCR products obtained. Similar considerations apply to attempts to shotgun-clone the DNA sample and analyse the resultant clones. So even if the same primer pair were used on several occasions, an enemy trying to detect the primer sequences would face an extremely difficult experimental barrier. Further mathematical and biochemical analysis would therefore be expected to prove that the primer pairs used in this technique are not analogous to a classic, single-use, cryptographical "one-time pad"[1].

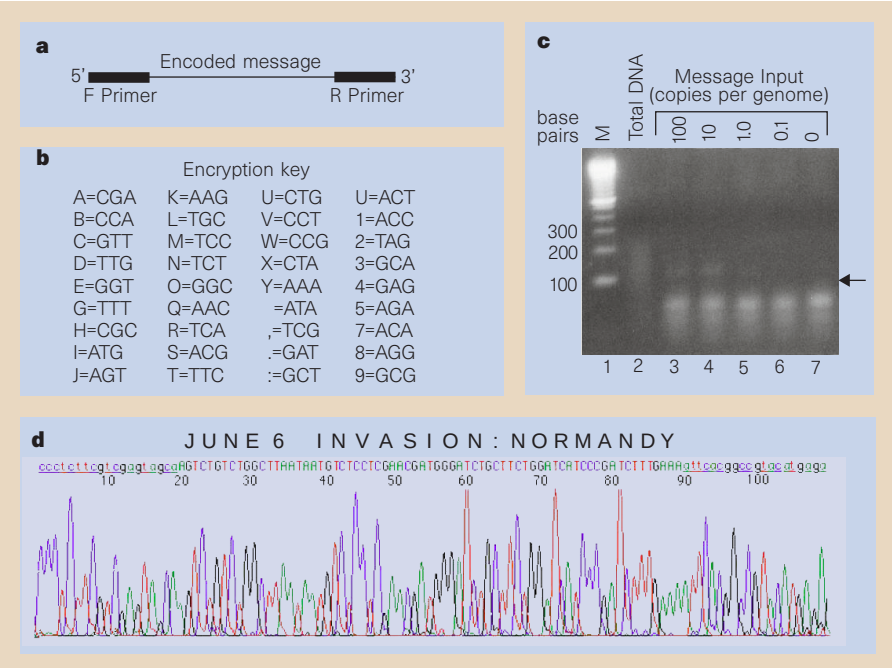

Figure 1 Genomic steganography. **a,** Structure of a prototypical secret-message DNA strand. F, forward; R, reverse. **b,** Key used to encode a message in DNA. **c,** Gel analysis of products obtained by PCR amplification with specific primers of microdots containing secret-message DNA strands hidden in a background of sonicated, denatured human genomic DNA. Message input in copies per human haploid genome is indicated, where 1.0 corresponds to 0.41 femtograms of secret-message DNA in 11 nanograms of human DNA. Lane 2 contains a message input of 100 (20-fold more total DNA than the microdots) and was not PCR amplified. M, 100-base-pair size markers. The gel was stained with ethidium bromide. The arrow indicates the PCR product seen in some lanes, below which primer–dimer bands can be seen. **d,** Sequence of the cloned product of PCR amplification, and the result of using the encryption key to decode the message. The DNA sequence determined for the encoded message is shown; the flanking primer sequences are in lower case. For details of the experimental methodology, see Supplementary Information.

Attempts by an adversary to use a subtraction technique to detect the secret-message DNA concealed within human DNA could be blocked by using a random mixture of genomic DNAs from different organisms as background. The intended recipient could still use the same procedures to amplify and read the secret-message DNA, even if ignorant of the random mixture composition, and even if the primers artefactually amplified a limited number of genomic sequences, because the encryption key would reveal which PCR product encodes a sensible message. This technique would also allow a single or duplicate microdots to be used to send individual secret messages to each of several intended recipients, each of whom would use a unique set of primers to amplify only his or her intended message.

To investigate the feasibility of this scheme, we synthesized a secret-message DNA oligodeoxynucleotide containing an encoded message 69 nucleotides long flanked by forward and reverse PCR primers, each 20 nucleotides long. We prepared concealing DNA that is physically similar to the secret-message DNA by sonicating human DNA to roughly 50 to 150 nucleotide pairs (average size) and denaturing it. We pipetted 6 μl of each solution containing 225 ng of treated human DNA, plus various amounts of added secret-message DNA, over a 16-point full stop printed on filter paper; it finally occupied an area about 20 times the size of the full stop. Excision of the printed full stops, each containing about 10 ng of DNA and with a cross-section that was about 75% larger than a full stop on this page, yielded DNA microdots.

Primers designed to amplify the secret-message DNA were used to perform PCR directly on DNA microdots, without prior DNA solubilization[3], and the products were analysed by gel electrophoresis (Fig. 1c). An unamplified sample containing secret-message DNA yielded only a faint continuous smear (Fig. 1c, lane 2). In contrast, amplification of DNA microdots containing either 100, 10 or 1 copies of the secret-message DNA per haploid genome (lanes 3–5) each yielded a single product of the expected size (arrow). No such product was

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detected using microdots containing either 0.1 (lane 6) or 0 (lane 7) copies per haploid genome, indicating a detection limit of about one secret-message DNA strand per haploid human genome. The amplified band in lane 4 of Fig. 1c (arrow) was excised, subcloned and sequenced. Use of the encryption key (Fig. 1b) to decode the resultant DNA sequence (Fig. 1d) yielded the encoded text, containing probably the most significant secret of the original microdot era: "June 6 invasion: Normandy" (Fig. 1d).

In preliminary experiments, microdots containing 100 copies of secret-message DNA per human haploid genome which had been attached using common adhesives to full stops in a printed letter, and posted through the US mail, yielded the correct PCR amplification product (data not shown). Our technique could therefore be used in a similar way to the original microdots: to enclose a secret message in an innocuous letter.

It should be possible to scale up the encoded message from the size of our simple example, perhaps by encoding a longer message in several smaller DNA strands. It should also be possible to use smaller microdots, which could be used for a variety of purposes, including cryptography and specific tagging of items of interest.

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1. Kahn, D. *The Codebreakers* (Scribner, New York, 1996).
2. Hoover, J. E. *Reader's Digest* **48,** 1–6 (April 1946).
3. Clayton, P. T. *et al. Arch. Dis. Child* **79,** 109–115 (1998).

Supplementary information is available on *Nature*'s World-Wide Web site (http://www.nature.com) or as paper copy from the London editorial office of *Nature*.

Neurochemicals aid bee nestmate recognition

The theory of kin selection[1], which revolutionized the study of social behaviour, requires the discrimination of relatives from non-relatives. Many animals possess this ability, but the underlying neurobiological mechanisms have not been studied. Here we provide evidence for the neurochemical modulation of nestmate recognition: treatment with octopamine agonists improves the discrimination of related nestmates from unrelated non-nestmates in honeybees.

We used a modification of a laboratory assay[2] that measures the probability that a group of five-day-old, laboratory-reared adult worker honeybees (*Apis mellifera*) will show aggression towards an introduced bee that is either a nestmate (from the group's natal colony) or a non-nestmate (from an unrelated colony). Group size was reduced from ten to three bees to make it possible for a single experimenter to inject all group members within a short period. Because the effects of octopamine injection are short-lived (about 60 min)[3], we treated bees with more persistent octopamine agonists: either 2,3-xylylaminomethyl-2′-imidazoline (XAMI)[4] or *N*′-(4-chloro-*o*-tolyl)-*N*-methylformamidine (DCDM)[5].

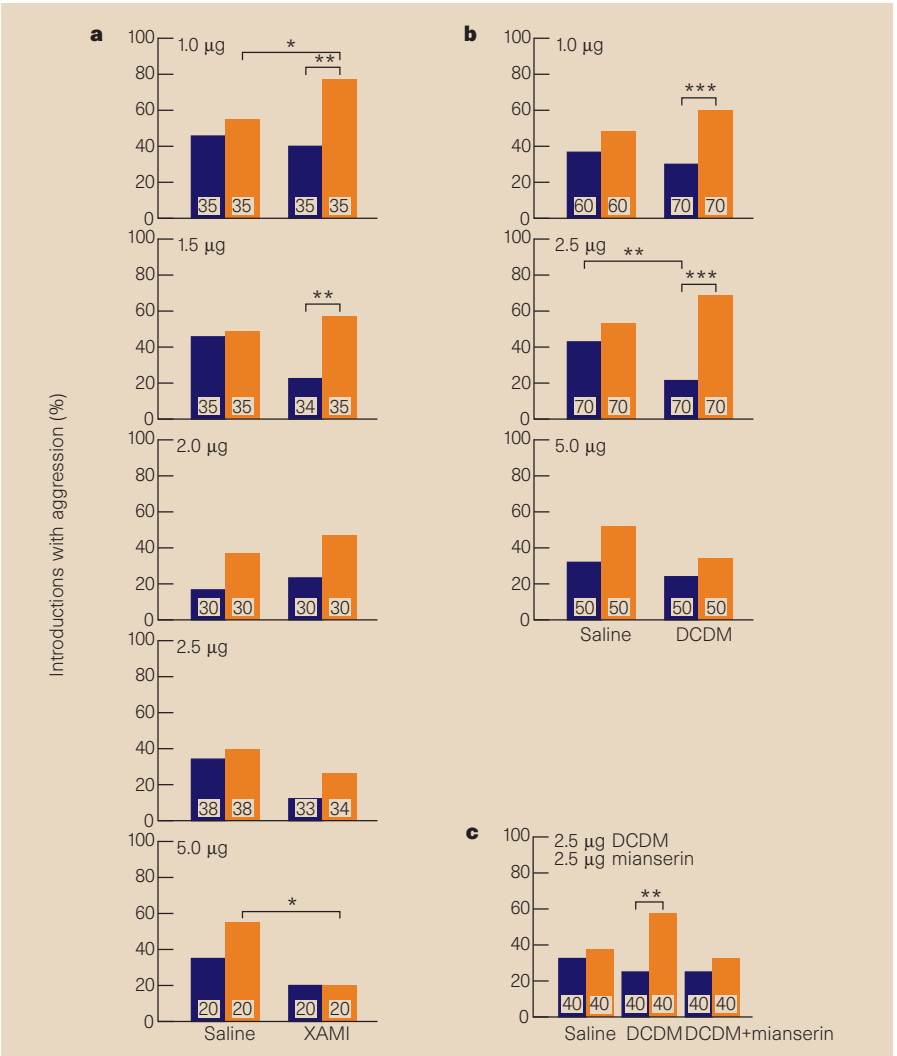

Figure 1 Effect of octopamine agonists on nestmate recognition in honeybees: **a,** XAMI; **b,** DCDM; **c,** DCDM plus mianserin (an octopamine antagonist). *P*-values are derived from two-way *G*-tests: **P*<<0.05; ***P*<0.01; ****P*<0.001. The number of introductions in each experiment is given in each bar. Purple bars, nestmate; orange bars, non-nestmate.

Bees given abdominal injections of 1.0 or 1.5 µg XAMI were significantly more likely to react aggressively towards non-nestmates than towards nestmates, but bees injected with saline were not (Fig. 1a). Comparisons with saline-injected bees suggest that the effect of XAMI was the aggregate result of two trends: decreased aggression towards nestmates and increased aggression towards non-nestmates. Only in one case was a trend significant by itself (aggression towards non-nestmates, with a dose of 1.0 µg; this was significant only at the $\alpha = 0.05$ level, and many statistical tests were performed). Higher doses of XAMI (2.5 and 5.0 µg) did not influence nestmate recognition but did cause a significant transient impairment of locomotor activity (data not shown), which might have interfered with the expression of aggressive behaviour. These results are consistent with depressed responses to both nestmates and non-nestmates in bees treated with the two higher doses (Fig. 1a). Octopaminergic agonists have been shown to have an effect on locomotion in other species[6].

To determine whether the results with XAMI reflected an octopaminergic process, we tested DCDM, an octopamine agonist from a different chemical family to XAMI. Results with DCDM were very similar to those with XAMI (Fig. 1b). Another indication of the specificity of the recognition effect is that it was eliminated when bees were treated with both DCDM and

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