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AN INFRARED MOTION DETECTOR SYSTEM FOR LOSSLESS REAL-TIME MONITORING OF ANIMAL PREFERENCE TESTS

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Automated behavioural observations are routinely used in many fields of biology, including ethology, behavioural ecology and physiology. When preferences for certain resources are investigated, the focus is often on simple response variables, such as duration and frequency of visits to choice chambers. Here we present an automated motion detector system that use passive infrared sensors to eliminate many drawbacks of currently existing methods. Signals from the sensors are processed by a custom-built interface, and after unnecessary data is filtered by a computer software, the total time and frequency of the subject's visits to each of the choice chambers are calculated. We validate the detector system by monitoring (using the system) and in the same time video recording mating preferences of zebra finches in a four-way choice apparatus. Manual scoring of the video recordings showed very high consistency with data from the detector system both for time and for frequency of visits. Furthermore, the validation revealed that if we used micro-switches or light barriers, the most commonly applied automatic detection techniques, this would have resulted in approximately 22% less information compared to our lossless system. The system provides a low-cost alternative for monitoring animal movements, and we discuss its further applicability.

Keywords: Motion detector – lossless monitoring – real-time – infrared sensor – mating preferences

INTRODUCTION

Monitoring the movements of experimental subjects in purpose-built setups is essential in many fields of biology, especially in ethology and physiology (e.g. learning processes, association and affiliation between individuals, preference studies) and behavioural ecology (e.g. investigating mating preferences). In these controlled laboratory studies, the subject is tested in a choice apparatus consisting of two or more choice chambers. These frequently open from a neutral (no-choice) chamber, and allow full or restricted access to different stimuli, including live individuals or some acoustic and/or visual cues (e.g. songs, different food items, or any objects of interest [2, 5, 7, 8]). In most studies, a stimulus is considered to be preferred if the subject

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spent most of the time in its vicinity (or most frequently visited it) relative to all the other stimuli presented during the preference test. Accurate monitoring of the movements of the subject during experimental sessions is therefore imperative and several detection methods have been developed and used for this purpose, albeit with various drawbacks. Below we review the most commonly used detection methods and we focus on their limitations, based on our research experiences.

One of the most frequently applied methods is manual scoring of the movement of the focal individual either real-time or using video-recordings of trials and later scoring behaviour (e.g. [5, 9, 10]). These methods, although do not need expensive and complicated setup, have the disadvantages of being time-consuming, susceptible to errors and exhausting by taking considerable man-power and concentration. In addition, scoring video recordings does not allow the researcher to get an immediate answer about the subject's preference after the trial (as often needed if further tests are based on the results of the preference test). These problems are exaggerated when sample sizes are large, and/or long experimental trials are applied to investigate preference.

Researchers therefore often use automated systems, which provide the benefits of real-time monitoring, taking no human effort and involving less opportunity for errors. For instance, mating preferences by female birds is often detected by micro switches (e.g. [4, 8]). These are small switches mounted beneath perches that are placed in the chambers of the choice apparatus. The micro switch closes each time the female lands on the given perch. This method is inexpensive, but setting up the system takes precision and testing. On the one hand, the micro switch has to close under the weight of the bird, on the other hand, it has to open when the bird leaves the perch. This means that the mechanics at each perch have to be fine-tuned and balanced perfectly. When the detected subject is a small animal (e.g. body mass of only a few grams, as in the case of passerine songbirds), finding the right balance may be challenging. In addition, mechanical systems tend to be more prone to failure and degrade more with time than systems including electronic parts only.

The application of photocells is very similar to micro-switches in that they are most frequently applied as light barriers to detect a bird's presence or absence on a given perch. The infrared emitter (IR LED) is mounted at one end of the perch that projects a light beam to an infrared receiver mounted at the opposite end of the perch (e.g. [1]). Whenever the infrared beam is broken, this switches a circuit and the signal is recorded. Photocells eliminate most of the problems of micro-switches, as being an opto-electronic device, they include no mechanical parts. However, care needs to be taken when aligning the emitter and receiver part of the photocell, and the system may be sensitive to ambient light conditions. Probably the most important drawback of micro-switches and light barriers is that a significant amount of valuable information might be lost during tests (see our results below); experimental trials likely include periods when the subject is in the vicinity of the stimulus (i.e. should be considered as expressing preference for the given stimulus), but does not trigger the micro-switch or light barrier (e.g. the bird lands on the ground or mesh instead, or other parts of the choice chamber). Also, these two perch-based detection methods are inapplicable in case of non-flying subjects, such as rodents or other terrestrial species. Although photocells (combined frequently with mirrors) can be installed at the entrance of each chamber, establishing the direction of movement through these photocells (i.e. whether the subject enters or leaves the given chamber) requires complicated solutions, such as two installed photocells at each gate, and this makes the system cumbersome.

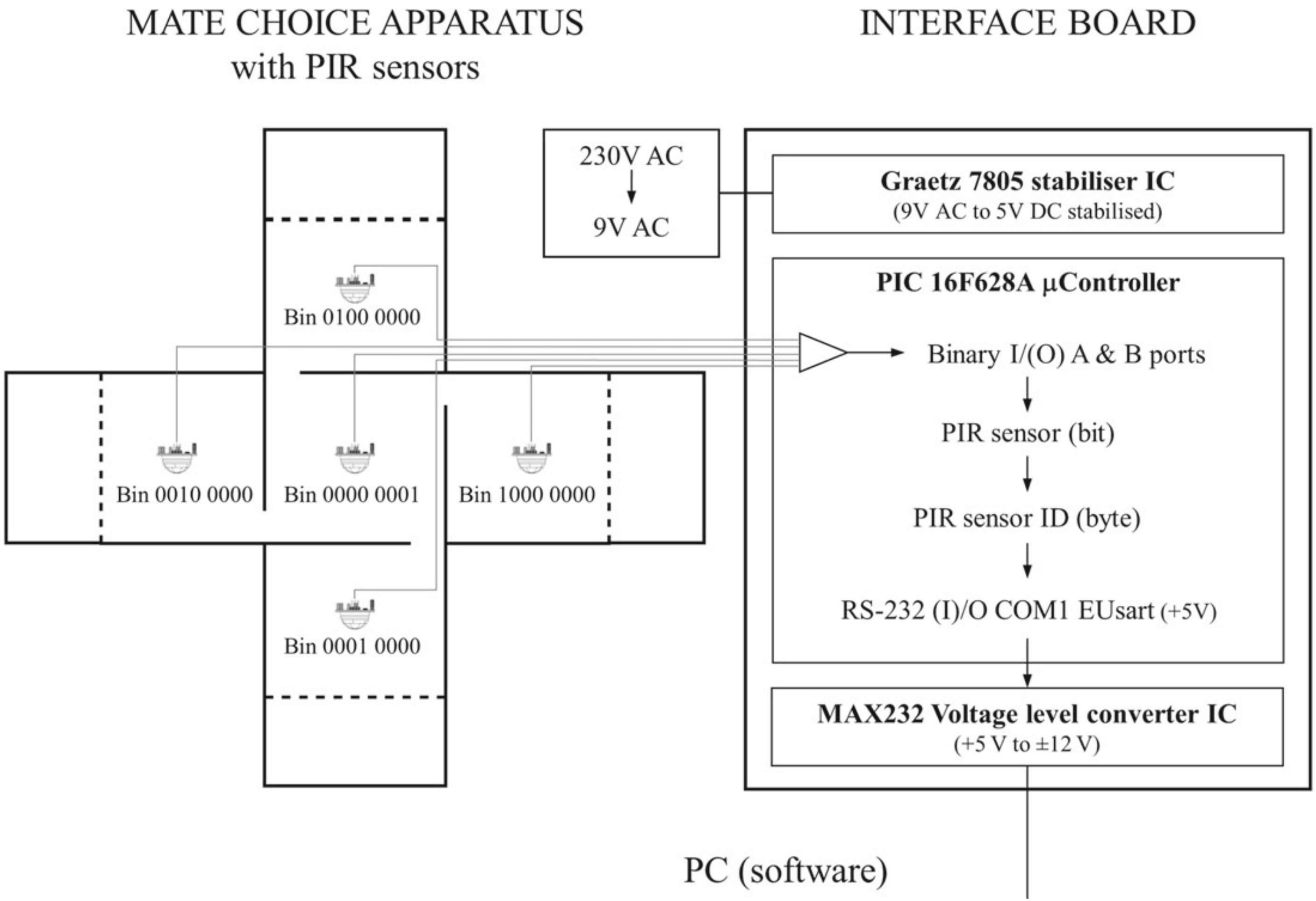
A more robust automated detection method for following the subjects' movements is provided by video-tracking (or object-tracking) software such as EthoVision XT or its successors from Noldus (Noldus Information Technologies, Wageningen, The Netherlands, [6]). These usually consists of camera(s) linked to a computer, preferably with recently developed hardware (in particular, a powerful graphic card) installed. The use of video-tracking software has the benefits of gaining very detailed information on the subjects' movements, and built-in analysing tools provide the opportunity to carry out in-depth analysis of behaviour during test sessions. Preference tests, however, often focus on simple response variables, such as duration and frequency of visits to the stimulus. The cost of the software license (but see "Predator", an open-source object-tracking software based on the recently developed Tracking-Learning-Detection method of Kalal et al. [3]) and/or the computer demands make video-tracking the most expensive option amongst the available possibilities.

Here we present a new automated detector system to monitor animal movements that is inexpensive, precise, robust and easy-to use. The motivation to build this system was that we needed a very stable motion detector system to monitor long (16 h) mate preference tests of a large sample (> 70) of zebra finch females (*Taeniopygia guttata*). Besides lossless, real-time information, our main requirements from the new system were robustness and reliability; we needed a system that can work faultlessly for an extended research period (for 6 months, continuous detection for 8 hours/day). Therefore, we built the system from inexpensive, commercially available, non-mechanic parts only, and used a very stable and simple programming environment to achieve low computing demands. Besides describing the main elements, here we validate the system by monitoring the movements of ten females during eight-hour preference tests. We compare data from the new motion detector system with manual scoring of video-recordings from the same trials.

MATERIALS AND METHODS

Setup of the infrared motion detector system

The hardware of our system includes three major elements: passive infrared sensors, an interface and a computer (Fig. 1). We used a similar mate choice apparatus to that of Swaddle and Cuthill [8] that consists of a neutral middle chamber and four choice chambers (Fig. 2). Thus, we used five commercially available passive infrared (PIR) sensors (no. 555-28027, Parallax Inc., Rocklin, CA, USA) with hemisphere shape for


MATE CHOICE APPARATUS
with PIR sensors
INTERFACE BOARD
230V AC
9V AC
Graetz 7805 stabiliser IC
(9V AC to 5V DC stabilised)
PIC 16F628A μController
Binary I/(O) A & B ports
PIR sensor (bit)
PIR sensor ID (byte)
RS-232 (I)/O COM1 EUsart (+5V)
MAX232 Voltage level converter IC
(+5 V to ±12 V)
Bin 0100 0000
Bin 0010 0000
Bin 0000 0001
Bin 1000 0000
Bin 0001 0000
PC (software)

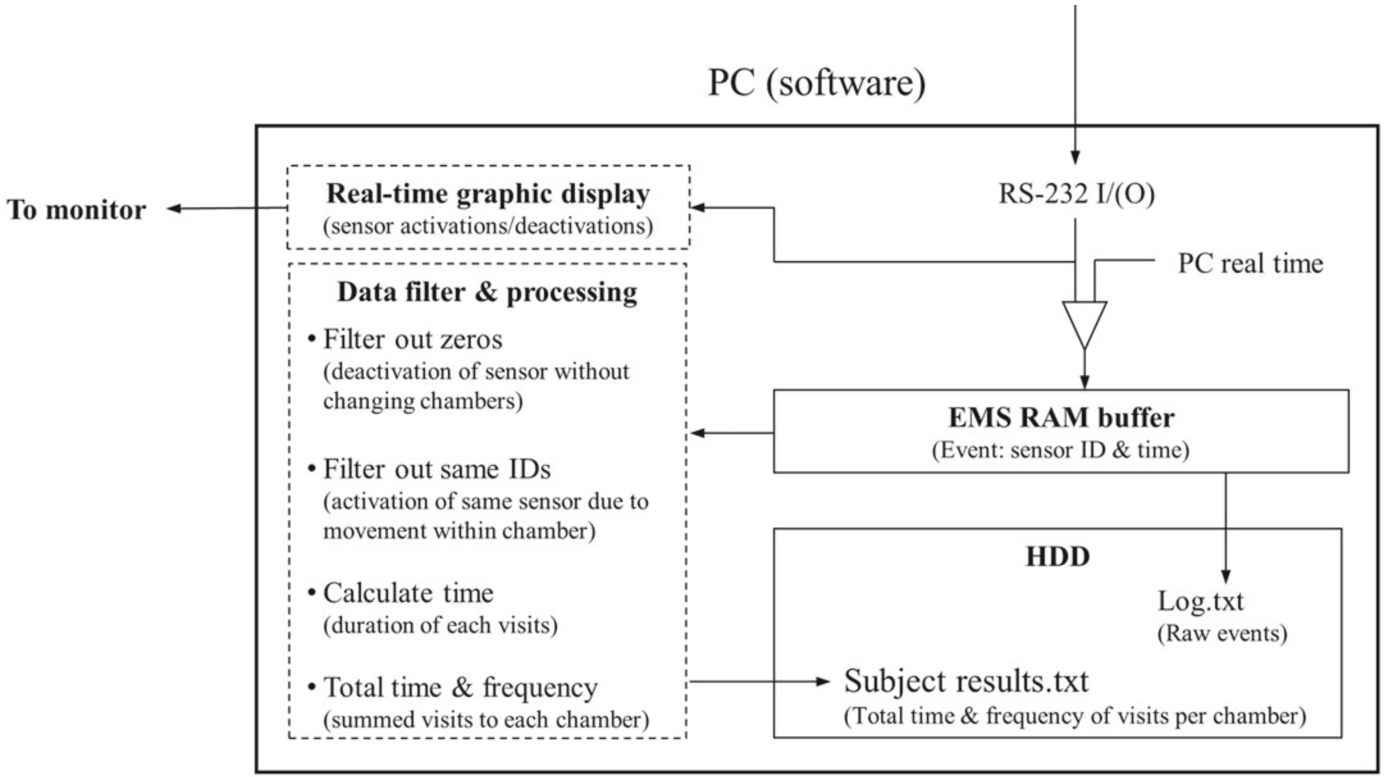


Fig 1. Not-to-scale schematic view of the four-way mate choice apparatus with the motion detector system. Dashed lines indicate wire mesh between stimulus compartments and choice chambers. The PIC microcontroller of the interface transformed 1/0 signals of each passive infrared sensor (PIR) to bytes corresponding to sensor IDs (IDs in binary are indicated under each PIR sensor), and following amplification, signals were processed by the computer software. Combined with the time stamp, the software saved each event as raw data, and displayed sensor activations and deactivations real-time on the monitor. At the end of a test session, a function of the software allowed to filter out unnecessary data points (i.e. deactivations of sensors and movements within choice chambers), and to calculate and save total time and frequency of visits to each choice chamber and the neutral chamber

180° view angle that provides wide detection range. The circuit boards of PIR sensors were protected by placing them in small plastic boxes with the sensor hemisphere uncovered. One PIR sensor was mounted in the centre of the top wall of each chamber including the neutral chamber, with the sensors' hemisphere facing down. The connection between the PIR sensors, the interface and the computer was through flexible, three-conductor cables.

We built a custom-designed interface, however, there are also commercially available I/0 boards (e.g. the Basic Stamp from Parallax Inc., Rocklin, CA, USA or various microcontroller platforms from Arduino, www.arduino.cc). Our interface included a PIC microcontroller (PIC 16F628A, Microchip Technology Inc., Chandler, AZ, USA; Fig. 1). The program of the microcontroller was compiled using PicBasic Compiler 1.45 (microEngineering Labs, Inc., Colorado Springs, CO, USA). From the PIC microcontroller's serial port connector (EUSART), the signal was enhanced using a level converter from +5V to ±12V signals (MAX232 RS-232 interface, Maxim Integrated Products, Inc., Sunnyvale, CA, USA; Fig. 1).

The interface was connected to the computer's serial port (RS-232 I/O). The computer software was compiled in Pascal, using Borland Turbo Pascal 7.0 (Borland Software Corporation, Austin, TX, USA). Our simple program runs in DOS so that it can run on any Windows operation systems from 9×, in a full-size DOS window. We used the OpenSource program DOSBox (v 0.74, developed by DOSBox Team) to emulate DOS and ×86 CPU using our system with Windows XP installed. (An alternative solution that offers wide compatibility is to adapt our software using a Pascal compiler built for the specific platform, such as various versions of Free Pascal, developed by Free Pascal Team.)

The main function of the program was fourfold; first, it displayed a schematic view of the choice apparatus on the monitor, and indicated when a certain PIR sensor was activated/deactivated real-time. This function allows the experimenter to monitor where the subject is at a given time and more importantly, whether the motion detector works faultlessly. To check correct functioning real-time, we occasionally compared the view on the monitor with that of a small security ccd camera mounted above the mate choice apparatus and connected to a digital video recorder and a television (see below).

The second function of the software was to save the time (using the internal system time of the computer) and the connection number (ID) of each PIR sensor as events, every time they became activated or deactivated due to the movement of the subject (and deactivation of the PIR sensor). This resulted in raw data that was logged from time to time for safety considerations, but since it included many unnecessary data points, it was processed further. PIR sensors kept activating and deactivating (deactivation took ca. 2 seconds if the subject did not move again) regardless of whether the subject left the chamber or moved within the chamber. Since from our perspective only those data points are relevant which indicate switches between chambers, the third objective of our program was to filter out two types of data points. First, all events with zero IDs were filtered out, as these indicate deactivation of a previously active PIR sensor while no new activation took place (because the bird stayed still).

Second, from consecutive events with the same PIR ID as that of the previous event only the first was kept and the others were deleted, as these indicated that the subject moved within a given chamber. During chamber switches, it is possible that more than one PIR sensors are activated simultaneously (for instance, the one in the chamber that the subject leaves and the one to which she arrives), therefore, we considered simultaneous sensor activations when writing the program. In such cases, the time spent in the previous chamber was calculated as the time between this sensor was first activated alone until the next sensor was activated alone (i.e. times during which simultaneous activations occurs are consistently added to times in previous chambers).

In addition, after an experimental test has been terminated, a function of the program allowed us to calculate the total time and the frequency of visits to each choice chamber. These summarized data, together with the total time of the test session, were saved as a text file output.

Further details on the detector system's hardware and software (including the executable) are available by e-mail request to the authors.

Birds and keeping conditions

Zebra finches were individually caged in 37×24×49 cm sized cages in four same-sex rooms (size of rooms: two with 3.4×3.4×2.4 m and two with 5.1×3.4×2.4 m). Individuals were separated visually, but not acoustically from each other within rooms, and were kept on a 10–14-hour regime (dark – light) using compact fluorescent light sources and digital time switches. Zebra finches were ringed by a numbered metal ring (A C Hughes, Middlesex, UK) for individual identification. Food and water were provided *ad libitum* every day, including experimental trials (i.e. during testing). We handled and experimented with the birds with care, and experimenting had no adverse effects on the birds. Our university's animal welfare guidelines were followed during maintenance and experimenting with the captive population.

Validation of the detector system

Validation was carried out by monitoring the mating preferences of ten experimental female zebra finches for eight hours each. Four different males were presented to each female in the stimulus compartments of the choice chambers during experimental trials (Fig. 2). During experimental trials, the female's movements were monitored by the detector system, and at the same time video-recorded by a small ccd camera (Videosec W-101, Euro Tech Corporation Kft, Hungary; mounted above the choice apparatus) that was connected to a digital video recorder (HDR-04RP, Hunt Electronic Co., Ltd., Taipei, Taiwan). Video recordings were manually scored for time and frequency of visits by one observer (ZSZ) using Observer program (version 2.01, Noldus Information Technology, Wageningen, The Netherlands) and these data were

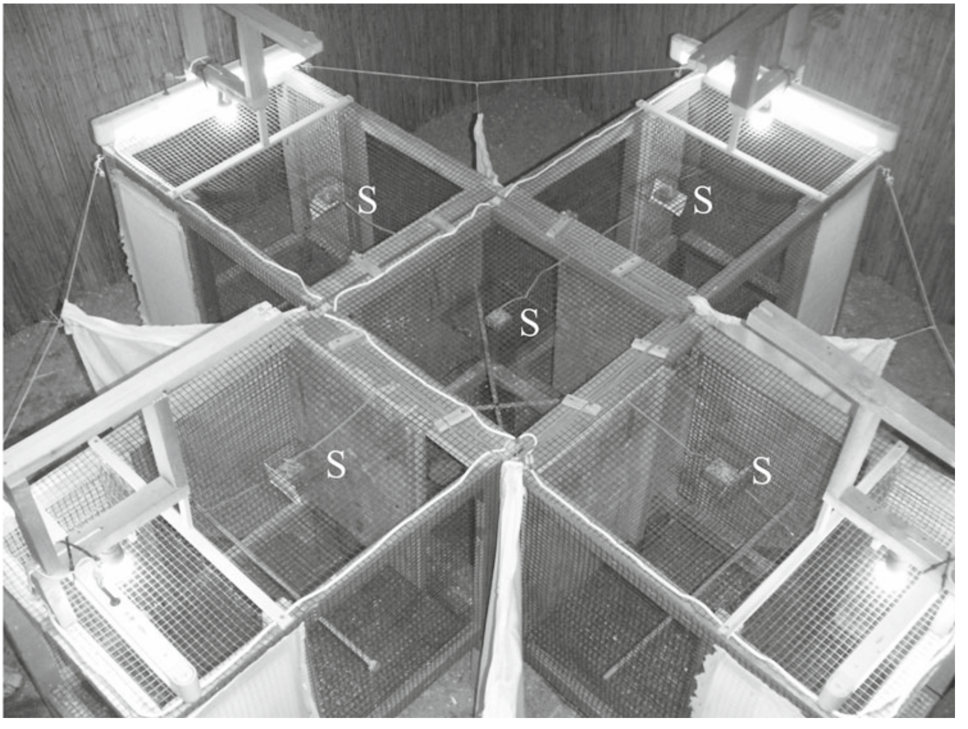


Fig 2. The four-way mate choice apparatus with five passive infrared sensors (S) installed. During tests, four males were presented in the stimulus compartments at the end of each choice chamber, separated by wire mesh. Passive infrared sensors were mounted in the centre of the top wall of each of the four choice chambers and the neutral (middle) chamber (i.e. in all chambers in which the female could move), facing downwards. To prevent sensor activation by the movements of stimulus males, the four sensors in the choice chambers were shaded by cardboard shields (10×5 cm) from the side of the stimulus compartment

correlated with those of the detector system. During manual scoring, we also monitored the female's movement between perches and other parts of the given chamber to assess the amount of data that would be lost by applying micro-switches or light barriers. We took into account statistical non-independence within our dataset as follows. Only times in (or frequencies of visits to) the four choice chambers were analysed for each female as the fifth time data (neutral chamber) could be calculated from the total time of test that has been kept fixed. In addition, the four time or four frequency data point pairs (manual vs. detector coding) for each female were correlated separately. Correlation coefficients were then averaged over the ten females both for time and for frequency (separately), and we provide the mean ± SE for these correlations.

RESULTS

We found very high correspondence between data provided by manual scoring of video recordings and the infrared motion detector system both for time and for frequency (Table 1; Pearson correlation coefficients, mean $r \pm$SE, times: 0.996 ± 0.002; frequencies: 0.943 ± 0.026).

Manual scoring of positions within chambers (whether the female was on the perch or off the perch) revealed that the ten experimental females spent in total 22.2 ± 5.4% of their test time off the perches during the trials, so this amount of information was detected by our system but would have been omitted by applying micro-switches or light barriers.

Table 1

Time and frequency of visits to each chamber in preference tests of ten female zebra finches, detected by manual scoring of video recordings (M) and the detector system (D)

ID	Detection method	Time in chamber (min)					Frequency of visits to chamber					Off-perch/ test time (min)
		U	R	B	L	N	U	R	B	L	N	
4	M	98	134	49	153	46	10	9	8	14	39	33/480
	D	101	133	50	153	42	12	13	10	18	48	
5	M	64	21	49	32	306	108	48	57	43	257	23/472
	D	71	21	52	33	295	177	57	65	52	337	
12	M	77	40	80	51	232	95	69	144	57	365	132/480
	D	81	49	91	51	208	92	59	139	71	336	
16	M	15	135	36	177	117	21	51	30	60	161	30/480
	D	18	137	35	178	112	22	54	36	73	158	
17	M	222	21	130	49	57	31	20	57	57	164	294/480
	D	225	23	135	49	48	31	19	70	61	150	
20	M	194	12	190	4	80	38	18	74	15	144	133/480
	D	197	12	194	5	73	29	19	60	17	116	
23	M	37	62	83	242	56	14	17	21	25	75	54/480
	D	38	63	83	241	56	12	18	28	30	79	
28	M	54	102	131	108	85	13	16	18	17	62	102/480
	D	58	103	133	100	86	13	17	23	20	70	
51	M	81	37	94	57	195	94	52	56	73	271	161/465
	D	89	39	97	59	179	77	42	60	72	183	
112	M	94	87	91	144	63	36	29	42	29	134	97/480
	D	99	88	90	144	61	34	31	48	39	129	

Chambers are identified based on the image of video recordings (top view of the choice apparatus as seen in Fig. 1) as follows: U – upper, R – right, B – bottom, L – left, N – neutral chamber.

DISCUSSION

We have developed a new automated motion detector system based on infrared motion sensors, and successfully applied the system to detect the movements of a small songbird species during mate preference tests. By manually scoring mating preferences of ten female zebra finches and correlating results with those of the detector system, we validated our system and found that it reliably and consistently detected the females' time in and movements between choice chambers, without any data loss.

We argue that this system has numerous advantages over others. Its simplicity and the use of inexpensive electronic parts make it cost-efficient and robust. Micro-switches/photocells seem to be the closest alternative to our system regarding complexity and price. However, since our system does not involve any moving mechanical parts, it is likely more stable than micro-switches. Indeed, in a recent experiment involving 63 females, each tested for their mating preferences for 16 hours (i.e. for 1008 work hours in total, using the detector system for eight hours almost every day for half a year), the system worked faultlessly and very reliably (Pogány et al. unpublished observation). In addition, as results of our manual scoring confirmed, applying micro-switches or photocells would have resulted in losing approximately a fifth of data due to females landing on other objects than the provided perches. This information loss can result in masking relatively weak patterns, especially when experimental trials are kept short.

Validation of our custom-built system revealed that the system provided almost consistent results to that of manual coding for time, whereas for frequency values, the correspondence was slightly weaker (although still highly consistent). The difference was most likely due to different scoring of two situations by the experimenter (manual scoring) and the detector system; short flies through the neutral, middle chamber and sitting in between chamber edges might have been coded differently.

In our system, we used passive infrared sensor modules, therefore sensors only detected the infrared radiation that the subject emits (hence 'passive'). We monitored the movements of zebra finches and even the heat that these well-insulated, feathered small passerine birds emitted was enough for the sensors to get correctly activated. Nevertheless, by using active sensors of various types (e.g. emitting in ultrasound or microwave length, and detecting the changes in reflection), the system can potentially be adapted to detect cold-blooded species. A great potential of our system in contrast with micro-switches and photocells is that it is applicable for terrestrial species. Since our system is modular, it is easy to adapt it to many experimental situations (for instance, using other types and/or different number of sensors).

Taken together, we propose that the system we developed offers a competitive alternative to the most frequently used detection methods. Besides providing real-time and lossless information, two further advantages of the system are its cost-efficiency and robustness. We suggest, therefore, that the application of this detector system should be considered in case of long-term experiments and/or when inexpensive setup is priority.

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