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Plasma cholinesterase characteristics in native Australian birds: significance for monitoring avian species for pesticide exposure

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Abstract

Cholinesterase-inhibiting pesticides are applied throughout Australia to control agricultural pests. Blood plasma cholinesterase (ChE) activity is a sensitive indicator of exposure to organophosphorus insecticides in vertebrates. To aid biomonitoring and provide reference data for wildlife pesticide-risk assessment, plasma acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities were characterised in nine species of native bird: King Quails (Excalfactoria chinensis), Budgerigars (Melopsittacus undulatus), White-plumed Honeyeaters (Lichenostomas penicillatus), Yellow-throated Miners (Manorina flavigula), Willie Wagtails (Rhipidura leucophrys), Australian Reed-Warblers (Acrocephalus australis), Brown Songlarks (Cincloramphus cruralis), Double-barred Finches (Taeniopygia bichenovii) and Australasian Pipits (Anthus novaeseelandiae). Plasma ChE activities in all species were within the range of most other avian species and all but one contained AChE and BChE; no AChE was present in King Quail, which has not previously been reported for any species. The lowest detectable plasma AChE activity was 0.10 mu mol min(-1) mL(-1) in Budgerigars and the highest was 0.86 mu mol min(-1) mL(-1) in Australian Reed-Warblers. BChE in the plasma ranged from 0.37 mu mol min(-1) mL(-1) in Double-barred Finches to 0.90 mu mol min(-1) mL(-1) in White-plumed Honeyeaters and Australian Reed-Warblers. The lowest proportion of AChE was found in Budgerigars (12.8%) and highest in Willie Wagtails (67.8%). No differences were detected in ChE activity at any time of day in Budgerigars and Zebra Finches (Taeniopygia guttata), although there was a significant difference in all ChE activity between seasons in Zebra Finches.

Keywords

pesticide, cholinesterase, exposure, characteristics, plasma, native, australian, birds, significance, monitoring, avian, species

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

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Plasma cholinesterase characteristics in native Australian birds: significance for monitoring avian species for pesticide exposure

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Abstract. Cholinesterase-inhibiting pesticides are applied throughout Australia to control agricultural pests. Blood plasma cholinesterase (ChE) activity is a sensitive indicator of exposure to organophosphorus insecticides in vertebrates. To aid biomonitoring and provide reference data for wildlife pesticide-risk assessment, plasma acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities were characterised in nine species of native bird: King Quails (*Excalfactoria chinensis*), Budgerigars (*Melopsittacus undulatus*), White-plumed Honeyeaters (*Lichenostomas penicillatus*), Yellow-throated Miners (*Manorina flavigula*), Willie Wagtails (*Rhipidura leucophrys*), Australian Reed-Warblers (*Acrocephalus australis*), Brown Songlarks (*Cincloramphus cruralis*), Double-barred Finches (*Taeniopygia bichenovii*) and Australasian Pipits (*Anthus novaeseelandiae*). Plasma ChE activities in all species were within the range of most other avian species and all but one contained AChE and BChE; no AChE was present in King Quail, which has not previously been reported for any species. The lowest detectable plasma AChE activity was 0.10 μmol min^{-1} mL^{-1} in Budgerigars and the highest was 0.86 μmol min^{-1} mL^{-1} in Australian Reed-Warblers. BChE in the plasma ranged from 0.37 μmol min^{-1} mL^{-1} in Double-barred Finches to 0.90 μmol min^{-1} mL^{-1} in White-plumed Honeyeaters and Australian Reed-Warblers. The lowest proportion of AChE was found in Budgerigars (12.8%) and highest in Willie Wagtails (67.8%). No differences were detected in ChE activity at any time of day in Budgerigars and Zebra Finches (*Taeniopygia guttata*), although there was a significant difference in all ChE activity between seasons in Zebra Finches.

Introduction

Organophosphate (OP) and carbamate insecticides are used routinely throughout Australia for the control of agricultural pests. Approximately 8000 t of these insecticides are applied annually (Radcliffe 2002). They are anti-cholinesterase (ChE) chemicals that inhibit esterases, including acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). AChE is an enzyme that hydrolyses the neurotransmitter acetylcholine (ACh) and thereby terminates cholinergic synaptic transmission (Walker and Thompson 1991). AChE has a high specificity for ACh and is inhibited at high substrate concentrations. BChE is a less specific esterase that has a higher affinity for the synthetic substrate, butyrylcholine, than it has for ACh and is not inhibited at high substrate concentrations (Thompson and Walker 1994). The most widely used group of anti-ChE insecticides are organophosphates (5000 t of active ingredients applied annually) and include parathion methyl, chlorpyriphos, dimethoate, profenfos and diazinon, while fenitrothion is primarily used for grain storage and locust control (Radcliffe 2002).

Because ChEs occur through the animal kingdom in both vertebrates and invertebrates, there is potential for poisoning in non-target species with organophosphate and carbamate pesticides (Hill 1992; Wilson *et al*. 1992; Fossi *et al*. 1996). The inhibition of AChE by such compounds causes a build-up of ACh in the synapse, leading to a disruption of normal functioning of the nervous system (Walker and Thompson 1991). Acute toxicity can result in death by respiratory or cardiovascular arrest, or both, and because cholinergic innervation of the body is nearly ubiquitous, sublethal exposures can lead to a range of biochemical, physiological and behavioural effects (Fryday *et al*. 1996; Grue *et al*. 1997).

The measurement of blood or tissue ChE activity is extensively used as a tool in diagnosing organophosphorus or carbamate insecticide exposure in animals (Fairbrother *et al*. 1991). It is an accepted diagnostic convention that whole-brain ChE inhibition >50% is indicative of anti-ChE exposure as the cause of death in avian mortality events (Ludke *et al*. 1975; Hill and Fleming 1982). Further, various physiological and behavioural changes have been associated with brain ChE

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activity when inhibited to 40–60% of unexposed levels (Hart 1993; Vyas *et al*. 1995; Fryday *et al*. 1996; Tamura *et al*. 2001). Though little is known about the function of ChEs in the plasma, their inhibition can be used to assess anti-ChE exposure and aid in diagnosing behavioural or physiological effects (Thompson 1991). Another value of plasma or serum ChEs as a biomarker lies in their usefulness for inferring exposure without lethally collecting the animal under assessment, and in this way has been used successfully to monitor exposure to agricultural chemicals in a variety of species (Hooper *et al*. 1989; Parsons *et al*. 2000; Strum *et al*. 2008). Since plasma ChEs are inhibited faster and more extensively than brain ChEs by anti-ChE compounds, exposure is more easily detectable. Further, owing to the more rapid recovery rate of plasma ChE activity, significant inhibition in the plasma indicates recent exposure (Gard and Hooper 1993). Similarly, a lack of plasma ChE inhibition following known exposure to anti-ChE compounds is strong evidence that the exposure level was below that which would cause effects (Goldstein *et al*. 1999).

Biomonitoring exposure to anti-ChE compounds on non-target avian species is particularly important when there is broad overlap of insect-control operations with high densities of birds. Such a scenario occurs in eastern Australia, where we have previously established that a variety of Australian native birds are exposed to fenitrothion during pesticide application for locust control (Fildes *et al*. 2006). Although the total application of pesticides by the Australian Plague Locust Commission varies from season to season depending on rainfall and other weather conditions, the average annual amount of fenitrothion applied for locust control from 1980 to 1998 was estimated to be 60–80 t year^{-1} (National Registration Authority 1999). Aerial spraying of locust bands and swarms has been the dominant form of application. Continual biomonitoring of anti-ChE exposure in non-target avian species before and after locust control or any agricultural spraying is essential to the assessment of ecological impacts.

Plasma ChE is known to have vary much, hence reference values are essential, and when known can be compared with values measured after application of pesticides, thereby enabling researchers to establish levels of exposure (Fossi *et al*. 1996). When reference values are unknown, inhibited ChE can be chemically reactivated, which also provides evidence of anti-ChE exposure and is a useful adjunct to comparative ChE analysis. However, an understanding of the appropriate assay conditions for individual species is required to measure ChE activity as well as obtain the most reliable results from reactivation. Despite this, there is little published information on ChE activity in species of Australian native birds, or Australian terrestrial vertebrates generally (Story and Cox 2001; Bain *et al*. 2004; Fildes *et al*. 2006; Buttemer *et al*. 2008). This lack of data must be remedied for the biomonitoring of sublethal pesticide effects to be incorporated into risk assessments of anti-ChE chemical exposure.

The primary aim of this investigation was to characterise the plasma ChEs, and establish the appropriate assay conditions, in nine avian species that co-occur with locust outbreaks. Fildes *et al*. (2006) reported that insectivorous locust-eaters were exposed to fenitrothion during locust control, as well as granivorous and honeyeater species, indicating that a variety of exposure routes exist for birds at locust spraying. Reference values for a variety of native birds will furnish baseline ChE data for performing anti-ChE exposure assessments in avian species following OP or carbamate application for locust control or any impact study following agricultural pesticide application. The nine study species consisted of birds from three orders: the granivorous King Quail (*Excalfactoria chinensis*; Galliformes) and Budgerigar (*Melopsittacus undulatus*; Psittaciformes), and seven species of Passeriformes, comprising the White-plumed Honeyeater (*Lichenstomus penicillatus*) and Yellow-throated Miner (*Manorina flavigula*), both of which feed on nectar, small insects and non-nectar carbohydrates, the insectivorous Australasian Pipit (*Anthus novaeseelandiae*), Brown Songlark (*Cincloramphus cruralis*), Australian Reed-Warbler (*Acrocephalus australis*) and Willie Wagtail (*Rhipidura leucophrys*), and the granivorous Double-barred Finch (*Taeniopygia bichenovii*) (Simpson and Day 2004; Higgins *et al*. 2006). King Quail were captive bred and maintained in outdoor aviaries at the University of Wollongong while the remaining birds were free-living and chosen on availability and ease of capture.

Our secondary aim was to examine the effect of time of day and time of year on plasma ChE in captive-bred Zebra Finches (*Taeniopygia guttata*) and captive-bred Budgerigars. Variation in plasma esterases has been attributed to diurnal and seasonal effects in several species from the northern hemisphere (Rattner and Fairbrother 1991). Hence temporal and seasonal variation in Australian species has the potential to influence the diagnosis of anti-ChE exposure. Therefore, an examination of temporal variation will benefit researchers during toxicological investigations using captive species. Zebra Finches and Budgerigars are appropriate representative study species as both are native to Australia and co-occur with locust outbreaks but are also available from commercial suppliers and are fairly easy to maintain in captivity.

Materials and methods

All but one of the nine species of birds used to characterise plasma ChEs (Table 1) were free-living; adult King Quails were captive-bred, purchased from a commercial supplier (Feathers, Fur and Fins, Warrowong, NSW) and maintained in captivity. King Quail were individually identified with metal leg-bands (L & M Bird Leg Bands Inc., San Bernardino, CA) housed (~16 birds per cage) at the University of Wollongong in outdoor aviaries ($3500 \times 2100 \times 2500$ cm), and provided with food and water *ad libitum*. Birds were allowed to adjust to caging conditions for at least 2 weeks before blood samples were taken. We controlled for the effect of age on ChE activity in the wild-caught species by only collecting blood samples from adult birds and while ChE activity may have been affected by sex, sample sizes were too small to test this. Therefore, sexes were pooled within species of free-living birds as well as in captive King Quail. Wild birds were caught at study sites in the Riverina region of southern central New South Wales (NSW), Australia, during September 2001, and in the Northern Rivers region of north-eastern NSW, during November 2001. These areas are primarily used for cattle grazing. Locust nymphs will often hatch from eggs buried in the ground in grazing pasture; hence the study sites were

Table 1. Plasma cholinesterase (ChE) activity from Australian avian species

Data for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are mean ± standard deviation; *n*, number of individuals sampled; n.d., not detected

Species	*n*	Enzyme Activity (mmol min^{-1} mL^{-1})		
		AChE	BChE	AChE as percentage of total ChE
King Quail	9	n.d.	2.23 ± 0.05	–
Budgerigar	6	0.089 ± 0.02	0.885 ± 0.19	9.1%
White-plumed Honeyeater	7	0.327 ± 0.06	1.130 ± 0.30	23%
Yellow-throated Miner	3	0.290 ± 0.07	0.926 ± 0.53	19.8%
Willie Wagtail	3	0.763 ± 0.09	0.508 ± 0.15	60.2%
Australian Reed-Warbler	1	0.860	0.901	48.8%
Brown Songlark	10	0.222 ± 0.05	1.06 ± 0.29	17.2%
Double-barred Finch	3	0.28 ± 0.02	0.41 ± 0.05	39.7%
Australasian Pipit	3	0.174 ± 0.01	1.075 ± 0.10	13.9%

selected because grazing country is the habitat most likely to be sprayed with pesticide during locust outbreaks. Other than aerially spraying fenitrothion to control locusts or the dipping of individual cattle with other anti-ChE compounds for tick control, pesticide application rarely occurs in these areas. There was no locust control or cattle dipping taking place in the study areas at the time of the study, so birds were unlikely to have been exposed to ChE inhibiting chemicals at the time of sampling. Wild birds were captured in mist-nets between 0600 and 1100 hours and were individually identified with Australian birds and bat banding scheme metal bands. For wild caught birds and captive King Quail, a 200 µL sample of blood (<1% of body weight) was taken before 1200 hours from a brachial vein by venipuncture with a 25-gauge needle and collected into heparinised microhematocrit tubes (Crown Scientific, Minto, NSW). Following centrifugation, plasma was extracted and frozen in liquid nitrogen. On return from the field to the laboratory, the samples were stored in a −80°C freezer until analysis. Samples from four species were analysed at the University of Wollongong and plasma samples from the remaining species were transported and analysed by the same individual at the Institute of Toxicology, Texas Tech University. The use of the same inter-assay standard confirmed that there was no difference between laboratories in the absolute ChE activities measured.

To examine effects of the time of sampling on plasma ChE activity, we obtained wild-type, captive-bred Zebra Finches and Budgerigars from a commercial supplier. All captive birds were individually identified with metal leg-bands, housed at the University of Wollongong in outdoor aviaries (3500 × 2100 × 2500 cm) with ~16 birds per cage, and provided with food and water *ad libitum*. Birds were allowed to adjust to caging conditions for at least 2 weeks before blood samples were taken. Zebra Finches ($n = 58$) and Budgerigars ($n = 10$) had a 200 µl blood sample taken once a week, over a 4-week period. A blood sample was taken on four different days at four different times of day: 0600, 1000, 1400 and 1800 hours, so that blood was sampled a total of 16 times from each individual. To examine the effect of season on plasma ChE activity, 58 plasma samples collected from Zebra Finches during the summer months of January and February were compared with 34 plasma samples collected during the winter months of June and July.

For all samples, plasma samples were assayed using the method of Ellman *et al*. (1961) as modified by Gard and Hooper (1993) for use in a 96-well spectrophotometric plate reader (BioRad, Crown Scientific, Minto, NSW) equipped with software for enzyme kinetic analysis (KC Junior, Bio-Tek Instruments, Winooski, VT, USA). Assay reagents were obtained from Sigma-Aldrich (Castle Hill, NSW). All the samples were assayed in triplicate for total ChE and AChE activities at 25°C for 3 min (readings taken at intervals of 13 s). Assay components were acetylthiocholine iodide (AThCh, 10^{-3} M final concentration; the ChE substrate), 5,5′-dithiobis (2-nitrobenzoic acid; 3.23×10^{-3} M), 0.05 M tris (pH 8.0) buffer and diluted enzyme with a total volume of 250 µL per microplate well. The assay was initiated by the addition of AThCh to all other components. AChE was differentiated from BChE by pre-incubation (5 min, before AThCh addition) with the specific BChE inhibitor, tetraisopropyl pyrophosphoramide (iso-OMPA, 10^{-4} M before addition of AThCh). BChE activity was calculated as the difference between total ChE and AChE activities. Horse serum, frozen in aliquots, provided a between-assay standard. Blank wells without added enzyme provided background colour formation. The increase in absorbance at 412 nm ($\Delta A\ min^{-1}$), corrected for blank, was converted to µmol AThCh hydrolysed per min per mL of plasma (or g of brain tissue) using the molar extinction coefficient 13 600 $M\ cm^{-1}$ (Ellman *et al*. 1961).

Characterisation was conducted on pooled plasma samples from at least five individuals of a species. A plasma pool was required, as the amount of plasma needed for characterisation is too large an amount to be collected from an individual bird without harming the animal. The appropriate plasma dilution factor, substrate and iso-OMPA concentrations were determined from the pooled plasma samples. The optimal plasma dilution was established so that total ChE activities achieved a $\Delta A\ min^{-1}$ value of 0.100 to 0.150. Iso-OMPA inhibition of BChE was determined over a range of iso-OMPA pre-incubation concentrations from 10^{-2} M to 10^{-12} M. The optimal concentration used in subsequent ChE determinations was that at which all BChE was inhibited, but at which AChE activity remained constant. Substrate affinity was determined by measuring AChE and BChE activities over a range of AThCh concentrations from 10^{-2} M to 10^{-6} M, using peak AChE activity to choose the appropriate assay concentration for both enzymes.

Once the plasma from each species was characterised using pooled samples, mean ChE activity levels for each species were determined using plasma samples taken from individual birds. Samples were diluted and assayed using the optimised concentration of assay components established through characterisation. The mean plasma ChE activities for all samples were calculated for each species.

The appropriate plasma dilutions for the assay were 5-fold in Double-barred Finches and Budgerigars, 20-fold in King Quails and 10-fold in all other species. In all species, except King Quails,

a concentration-dependent decrease in ChE activity occurred as iso-OMPA concentration increased and a plateau of inhibition was reached at which BChE was inhibited and AChE activity remained. An iso-OMPA concentration of 10^{-4} M was chosen for use in all species with the exception of King Quails, as this concentration led to activities being within the plateau of BChE inhibition. Variable quantities of residual AChE activity were demonstrated at the chosen plateau iso-OMPA concentration.

King Quails were notable for their lack of measurable activity at iso-OMPA concentrations above 10^{-5} M, suggesting a lack of plasma AChE. The characterisation of King Quail plasma was repeated four times with four different pooled plasma samples and the result was consistent. Consequently, the lack of a plateau in King Quails precluded the use of iso-OMPA as a selective inhibitor of BChE in order to isolate AChE, therefore only total ChE could be measured in this species.

The optimised iso-OMPA concentration in all other species was used to assess substrate affinity. The AThCh concentration that led to maximum AChE activity in Australian Reed-Warblers and Double-barred Finches was 316 mM. In all other species apart from King Quails, optimum AThCh concentration was 100 mM. Plasma ChE in King Quails did not reach full maximal activity levels at concentrations of AThCh or BThCh up to 10^{-2} M.

Effects of sampling time on ChE were assessed for normality using the Shapiro-Wilk test. All data were normally distributed, so that effect of time of day in captive Zebra Finches and captive Budgerigars were examined using an ANOVA for repeated-measures, whereas effects of time of year were examined using a Student's *t*-test. The significance level was $P < 0.05$; results are expressed as means ± s.e. Analyses were performed using JMP statistical software (Version 5.1, SAS Institute Inc., Cary, NC). Statistical procedures follow those outlined in Zar (1999).

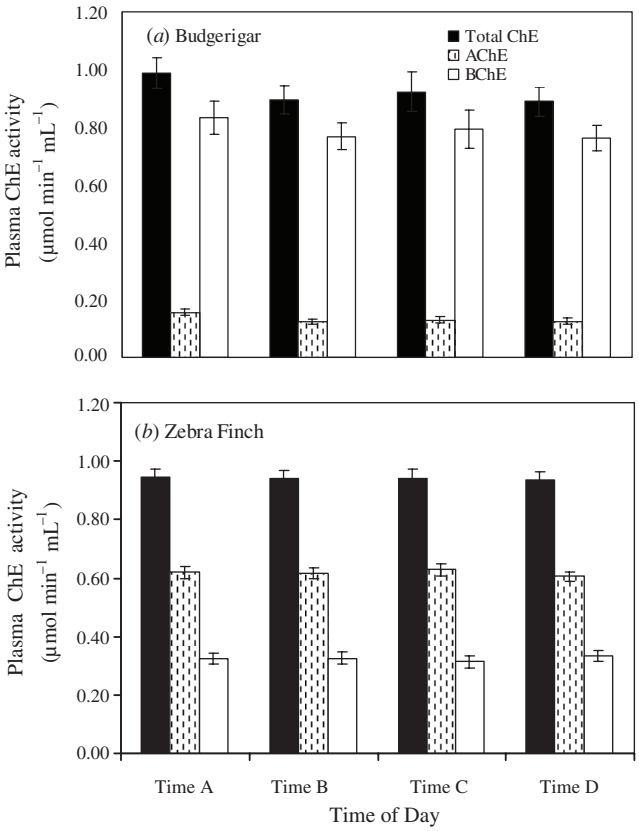

Fig. 1. Mean plasma cholinesterase (ChE) activity at four different times of day (± s.e.) in (*a*) Budgerigars ($n = 10$ individuals) and (*b*) Zebra Finches ($n = 58$ individuals). There were no significant differences in any enzyme activity at any time point.

Results

The variation in mean plasma AChE activity across species ranged from 0.089 μmol min^{-1} mL^{-1} in Budgerigars to 0.860 μmol min^{-1} mL^{-1} in Australian Reed-Warblers (though sample size of latter was only one) (Table 1). BChE in the plasma ranged from 0.414 μmol min^{-1} mL^{-1} in Double-barred Finches to 1.13 μmol min^{-1} mL^{-1} in White-plumed Honeyeaters. White-plumed Honeyeaters, Yellow-throated Miners and Budgerigars had a lower proportion of AChE than BChE in the plasma, the latter displaying the lowest measurable activity, at 0.089 μmol min^{-1} mL^{-1} (9.13% AChE) (Table 1). Plasma ChE in Willie Wagtails had the highest percentage of AChE at 60.2%.

Plasma ChE activity in Zebra Finches did not vary significantly with time of day. Total ChE and BChE activity in this species changed between morning and evening by as little as 0.5% and 2.3% respectively (Fig. 1*a*; total ChE $P > 0.07$, BChE $P > 0.1$). AChE displayed slightly greater variation but did not change significantly throughout the day ($P > 0.3$). Plasma ChE in Budgerigars varied more than in Zebra Finches. Although the morning samples appeared to be generally higher in all ChE activities than the evening samples (Fig. 1*b*), this variation was not statistically significant for any ChE activity, nor at any time of day in this species ($P > 0.2$). Time of year, however, did have an effect on all plasma ChEs in Zebra Finches (Fig. 2), with plasma activity significantly higher during the summer months than during winter ($P < 0.001$).

Discussion

The defining characteristics of AChE include the hydrolysis of AThCh, resistance to the selective inhibitor iso-OMPA and inhibition at high concentrations of AThCh (Radic *et al.* 1993). By this definition, all native bird plasma samples, excepting King Quails, contained AChE, an iso-OMPA resistant fraction, and BChE, an iso-OMPA sensitive fraction. In all species except King Quails, there was a concentration-dependent inhibition of a portion of the total ChE activity, with iso-OMPA as inhibitor, and there was little difference in iso-OMPA concentrations where the plateau of inhibition was reached. Unlike the rest of the species, in King Quails all ChE in the plasma was inhibited at low concentrations of iso-OMPA, with no demonstration of residual AChE activity that was resistant to the selective inhibitor. The hydrolysis of AThCh was not inhibited at high concentrations of AThCh. By definition there was no measurable AChE in the plasma of captive-bred King Quails, only BChE.

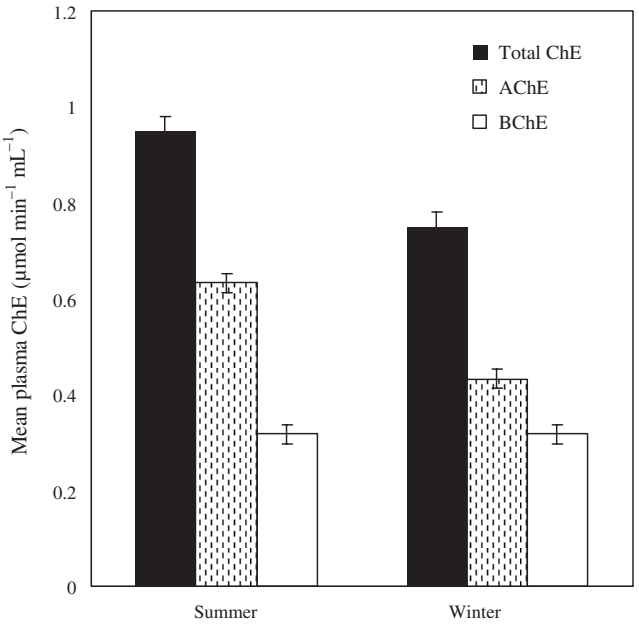

Fig. 2. Seasonal changes in mean plasma cholinesterase (ChE) activity in Zebra Finches (± s.e.; *n*: summer = 58 individuals, winter = 34 individuals). All plasma ChE activities in winter were significantly lower than in summer.

Butyrylcholinesterase has been shown to be predominant in the plasma of most of the avian species studied to date (Claudie *et al*. 2005). Our study confirms this trend as in five of the seven species characterised, the BChE fraction in plasma was higher than AChE. The lowest concentration of AChE, 9.13% of total ChE, was found in Budgerigars and this was similar to values reported by Gard and Hooper (1993) in the Eastern Bluebird (*Sialia sialis*) and Common Starling (*Sturnus vulgaris*). In the present study, BChE activity in White-plumed Honeyeaters, Yellow-throated Miners, Australasian Pipits and Brown Songlarks contributed to over 75% of plasma ChE; high concentrations of BChE have also been reported in the plasma of Barn Owls (*Tyto alba*) and Rock Doves (*Columba livia*) (Thompson *et al*. 1991). Although very low AChE activity has been found in several species, there have been no previous investigations reporting a lack of AChE activity in avian plasma as we have demonstrated in King Quails. Interestingly, the lowest reported AChE fraction in birds was found in another galliform species, the domestic chicken (*Gallus domesticus*), with AChE contributing only 3% to total ChE activity (Hooper 1988). In Tawny Owls (*Strix aluco*) and Red-winged Blackbirds (*Agelaius phoeniceus*) AChE only accounted for 6% of plasma ChE (Wolfe and Kendall 1998; Claudie *et al*. 2005).

There is some indication in the literature that related species have similar fractions of ChE in the plasma. In 20 European raptor species there was wide interspecies variation in ChE reported (Claudie *et al*. 2005), but with a trend towards a relationship between ChE activity and phylogenetic classification. Westlake *et al*. (1983) examined, among other enzymes, brain AChE in 47 bird species, and plasma AChE in 19 species and showed, despite interspecies variation, discernible familial trends. White-browed Woodswallows (*Artamus superciliosus*) and Masked Woodswallows (*A. personatus*) of Australia, had similar fractions of ChE in the plasma, of 68% and 60% AChE respectively (Fildes *et al*. 2006), and Snowy Egrets (*Egretta thula*) and Little Blue Herons (*E. caerulea*) both had 75% AChE in plasma (Parsons *et al*. 2000). Clearly we have characterised too few species as yet to evaluate discernable phylogenetic trends in Australian native birds and to do this is beyond the scope of the present study. However it is interesting that White-plumed Honeyeaters and Yellow-throated Miners are both old Australian endemic species from the family Meliphagidae (Parvorder Corvida) and although actual ChE activity was not similar in these species, they do demonstrate similarity with regard to their ChE fractions in plasma (Table 1). Both species had plasma consisting of ~28% AChE and 72% BChE. Likewise, Double-barred Finches had similar plasma ChE fractions to free-living Zebra Finches, which are within the same genus (Fildes *et al*. 2006). The plasma in the latter consisted of 53% AChE and 47% BChE, and, in the present study, Double-barred Finches were found to have plasma consisting of 42% AChE and 58% BChE.

This study found no evidence that plasma ChE activity varies with time of day in Zebra Finches or Budgerigars but did distinguish a difference between summer and winter in all ChE activities in Zebra Finches. ChE activity in winter was 68% of activity during the summer months. The latter result is not unexpected as seasonal differences in serum and plasma ChE activity have been reported for a wide range of avian species (Hill and Murray 1987). There is little information available on circadian rhythms and seasonal variation in ChE activity and the existing information varies among species examined (Garcia-Rodriguez *et al*. 1987; Rattner and Fairbrother 1991). As much as a 22% difference in basal activity has been observed at different times of day in Northern Bobwhite (*Colinus virginianus*) hens (Rattner and Fairbrother 1991), whereas a marked diurnal variation in plasma carboxylesterase, an enzyme closely related to ChE, was detected in Common Starlings (Thompson *et al*. 1988). ChE in blood samples taken over a 24-h period from Common Buzzards (*Buteo buteo*) underwent circadian rhythms, but samples from Eurasian Eagle-Owls (*Bubo bubo*) in the same study did not (Garcia-Rodriguez *et al*. 1987). The lack of daily variation in plasma activity in captive Zebra Finches and Budgerigars in the present study allows for greater flexibility in sampling regimes and demonstrates a further advantage of using these species in captive studies as representative Australian native species. However, this information should not be extrapolated to free-living impact studies.

The baseline plasma ChE estimates in free-living species presented here will provide a useful comparison but would not be a substitute for an impact study that compares samples collected before pesticide application with those collected after. Further, the variation between species described in the literature and as found here precludes information from one species being extrapolated with certainty to any other. Therefore, important considerations for designing wildlife monitoring programs and interpreting data include whether or not birds have been at risk of exposure to anti-ChE compounds, the time since exposure, age and sex of birds, time of day and season.

Interestingly, free-living Zebra Finches (Fildes *et al*. 2006) had plasma ChE activities 42% lower than the captive Zebra

Finches in the present study measured at the same time of year (Fig. 2). This difference in activity is likely to be a result of several factors. In wild-caught Zebra Finches, individuals of a certain age or sex may have been over-represented among those sampled, or captive-bred birds may have differed from wild birds in reproductive or nutritional status. All of these factors have been shown to affect avian ChE activities (Rattner and Fairbrother 1991; Gard and Hooper 1993) and natural sources of variation would be vastly different in the wild than in the aviary, especially considering the low variation in diet together with the consistency of feeding times in captive birds. Such intraspecies variation further highlights the need for reference values and the necessity for reduced variation in methodology and procedures so baseline measurements can provide the greatest comparability of information (Fairbrother and Bennett 1988).

In conclusion, the variability in plasma ChE activity between species underscores the benefit of characterising AChE before using the enzyme in monitoring schemes. Baseline measurements of ChE activity in Australian native bird species have to date been virtually absent in the literature. The present contribution begins to fill the void of ChE information, aiding field investigations and biological monitoring of non-target wildlife, providing for diagnostic evaluations where birds are at risk of exposure to anti-ChE compounds.

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