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# Modelling Genetic Benefits and Financial Costs of Integrating Biobanking into the Captive Management of Koalas

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**Table S1.** Captive holding/management costs for koalas used in cost modelling presented in this study. The table includes each itemized cost including a description. The costs are presented per individual animal. Itemized costs are presented as either fixed or variable. The frequency at which individual cost components recur in long-term cost modelling is also shown (based on the effective life of depreciating assets as per the Australian Taxation Office: https://www.ato.gov.au/law/view/document?docid=TXR/TR20195/NAT/ATO/00003 access date: 29 March 2022). All dollar amounts are shown in Australian currency (A$).

| Costs for conventional koala captive management used for modelling | Description | Costs (per individual animal) | Fixed or variable | Recurrent costs frequency (year intervals)* |
|---|---|---|---|---|
| Annual captive holding cost per individual animal | Annual average holding cost per individual koala (including veterinary, husbandry, eucalypt plantation management, genetic management and staff). Value based on expert practitioner knowledge from Steve Johnston and the former Dreamworld Life Science Manager. | $12,000 | Variable | 1 (every year) |
| On-site infrastructure/facilities costs | Note: does not include infrastructure and facilities costs. Koala enclosure. On-site infrastructure. 4-8 koalas. Between $60-80K. Year 1. | $11,667 | Variable | 20 |

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|---|---|---|---|---|
| | Per individual cost is based on the median number of 6 koalas applied to the median cost of $70k. | | | |
| **Total Set-up** | n.d | **$23,667** | n.d | n.d |

**Table S2**. Costs specific to the integration of biobanking and assisted reproductive technologies used in the cost modelling presented in this study. The table includes each itemized cost including a description. Itemized costs are presented as either fixed or variable. The Year 1 costs are shown as well as the frequency at which these same individual cost components recur in long-term cost modelling (based on the effective life of depreciating assets as per the Australian Taxation Office: https://www.ato.gov.au/law/view/document?docid=TXR/TR20195/NAT/ATO/00003; access date: 29 March 2022). All dollar amounts are shown in Australian currency (A$).

| Component of biobanking system for backcrossing | Description | Year 1 Costs | Fixed or variable | Recurrent costs frequency (year intervals) |
|---|---|---|---|---|
| Anesthetic machine – isoflurotec | Required to sedate koalas during semen collection or assisted reproductive procedures. | $10,000 | Fixed | 10 |
| Phase contrast microscope | Used to assess and manage semen samples. | $15,000 | Fixed | 10 |
| Electroejaculation equipment and probes | Required to collect sperm from koalas in the field and in the hospital. | $10,000 | Fixed | 6 |
| Laparoscope | Required for ultrasound guided oocyte aspiration (oocyte collection) and embryo transfer. | $20,000 | Fixed | 10 |
| Benchtop incubator (GERI) and culture media | Required for oocyte and embryo culture. | $50,000 | Fixed | 7 |
| Stereoscope and stage warmer | Required for intracytoplasmic sperm injection. | $12,000 | Fixed | 10 |
| Anti-vibration table, micromanipulator controllers and consumables | Required for intracytoplasmic sperm injection. | $78,000 | Fixed | 10 |
| Air filtration unit (Coda) plus filters | Required for intracytoplasmic sperm injection and embryo culture. | $5,000 | Fixed | 10 |
| Portable incubator for oocytes and embryos | Required for transport of oocytes and embryos. | $5,000 | Fixed | 7 |
| Fridge/freezer for media, extenders | Required for frozen storage. | $800 | Fixed | 10 |
| Consumables | e.g., Liquid Nitrogen, hormones, french straws. | $54,000 | Fixed | n.d |
| Biobanking equipment & sample quality assurance consumables | e.g., sterile straws/vials, straw sealant balls, diluent reagents, sperm chromatin stability assays, viability stains/activation reagents, semen and cryodiluent filters, and liquid nitrogen. | $15,610 | Fixed | n.d |
| Dewars and freezers | Required for the frozen and chilled storage of sperm. | $50,000 | Fixed | 10 |

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| Additional Founder collection | Required for the supplementary collection of founder sperm. | $20,000 | Fixed | n.d |
| Total Set-up | n.d | $345,410 | Fixed | n.d |
| Backcross costs (labor) total / yr | Reproductive technician (1.0x full-time equivalent) (to perform all backcross procedures using either backcross tool; intracytoplasmic sperm injection or artificial insemination; to perform all sperm cryopreservation and thawing): $94,651.<br><br>Scientific Officer (0.5 full-time equivalent) (to oversee all reproductive technology procedures , ovary culture, oocyte maturation, intracytoplasmic sperm injection, and sperm cryopreservation): $70,000. | $164,651 | Fixed | 1 (every year) |

We adjusted previous iterations of the genetic backcrossing model presented for amphibians [1,2] and other marsupial species [3] to account for the unique challenges and characteristics of *P. cinereus* captive management, including reproductive complexity, life history traits, increased holding costs and requirements, generation length, backcross frequency, and effective population sizes ($N_e$).

**Table S3**. Genetic modelling assumptions and parameters for captive colonies of koalas. Includes general assumptions for genetic modelling already presented in previous iterations [1–3], as well as species-specific parameters used to adapt this modelling approach to suit the koala.

| **Genetic modelling assumptions and parameters for captive colonies of koalas (*Phascolarctos cinereus*). Includes general assumptions for genetic modelling already presented in previous iterations [1–3], as well as species-specific parameters used to adapt this modelling approach to suit the koala.** |
|---|
| Model assumes *P. cinereus* founder males and females in the colony have $F_0 = 0$. |
| The captive colony would initially contain one live female per founder male (drawn at random from *P. cinereus* source populations). Under backcrossing scenarios, males produced each generation are redundant after founder sperm are banked from the $F_0$ generation. |
| Colony census numbers ($N$) are managed at each generation to achieve the target $N_e$ ($N_e$ assumed constant). |
| An average 1:1 sex ratio is assumed and achieved by colony management. An average of one female offspring per female contributed to subsequent generations (via management of the colony). |
| Random pairing of males and females occur each generation during back-crossing. Live colony pairings are uncontrolled (to be consistent with assumptions for published $N_e$ values). |
| Each generation produced would entirely replace previous captive generation with no overlap. |
| Females in each non-backcrossed generation have the same variability in offspring numbers as do wild females. |

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| At each backcrossing event one fertile female offspring is retained from each female in the colony. Male offspring are redundant and removed. |
| All biobanked founder males are unrelated ($F_0 = 0$). |
| Novel species-specific genetic modelling assumptions and parameters for captive colonies of *P. cinereus* |
| The frequency of *P. cinereus* females in the captive colony successfully breeding at a mean generation length of ~7 years has the same variability as wild females. |
| All *P. cinereus* females in the captive colonies are optimally sexually mature at 2 years of age, enabling artificial insemination and intracytoplasmic sperm injection to be performed on captive females at the appropriate mean generational interval of ~7 years. Male *P. cinereus* are not sexually mature until ~2 years of age necessitating their collection from the wild when mature or keeping in captivity until at least 2 years of age. |
| These values represent the *ex situ* age at optimal breeding for each species based on husbandry and published values [4] and a published assumed ~7-year generation length [5]. Here we assume that all individuals would be optimally sexually mature and breeding by this age. |

**Table S4.** Costs for the integration of biobanking into the nodes presented in Figure 2. The table includes each itemized cost including a description. Itemized costs are presented as either fixed or variable. The Year 1 costs and on-going costs are shown. These costs are specific to the collection and storage of koala founder sperm and have been isolated from the detailed costs in Table S2.

| Itemized costs for biobanking systems at each location across the mapped node network (Figure 2) | Description | Year 1 costs | On-going costs | Fixed or variable |
|---|---|---|---|---|
| Anesthetic machine – isoflurotec ** | Required to sedate koalas during semen collection or assisted reproductive procedures. | $10,000 | n.d | Fixed |
| Electroejaculation equipment and probes | Required to collect sperm from koalas in the field and in the hospital. | $10,000 | n.d | Fixed |
| Fridge/freezer for media, extenders | Required for frozen storage. | $800 | n.d | Fixed |
| Consumables | e.g., Liquid Nitrogen, hormones, french straws. | $54,000 | n.d | Fixed |
| Biobanking equipment & sample quality assurance consumables | e.g., sterile straws/vials, straw sealant balls, diluent reagents, sperm chromatin stability assays, viability stains/activation reagents, semen and cryodiluent filters, and liquid nitrogen. | $15,610 | n.d | Fixed |
| Dewars, dry shippers and freezers | Required for the frozen and chilled | $50,000 | n.d | Fixed |

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|---|---|---|---|---|
| Reproductive technician | storage of sperm and sample transport. (0.5x full-time equivalent) (to perform all sperm cryopreservation and thawing). | $47,326 | $47,326/yr | Fixed |
| Total Set-up | n.d | $187,736 | $47,326/yr | Fixed |

**Table S5.** GPS locations of each organization revealed in the online search for the proposed integration of biobanking and assisted reproduction for the koala across the node network presented in Figure 2.

| Node Organization | Latitude | Longitude | State of Australia |
|---|---|---|---|
| Friends of the Koala | -28.820818 | 153.302155 | New South Wales |
| Port Macquarie Koala Hospital | -31.443247 | 152.919647 | New South Wales |
| Port Stephens Koala Hospital | -32.76509 | 152.11442 | New South Wales |
| Taronga Conservation Society Wildlife Hospital (Dubbo) | -32.273024 | 148.586592 | New South Wales |
| Taronga Conservation Society Wildlife Hospital (Sydney) | -33.843177 | 151.239956 | New South Wales |
| Moggill Koala Rehabilitation Centre | -27.576891 | 152.875033 | Queensland |
| Currumbin Wildlife Hospital | -28.139845 | 153.48499 | Queensland |
| Australia Zoo Wildlife Hospital | -26.834896 | 152.961458 | Queensland |
| RSPCA Queensland Wildlife Hospital (Wacol) | -27.586065 | 152.922088 | Queensland |
| RSPCA Queensland Wildlife Hospital (Eumundi) | -26.471882 | 152.963704 | Queensland |
| Hidden Vale Wildlife Centre | -27.71638 | 152.463288 | Queensland |
| Ipswich Koala Protection Society | -27.587849 | 152.595372 | Queensland |
| Werribee Open Range Zoo Koala Hospital | -37.922345 | 144.667319 | Victoria |
| Healesville Sanctuary Australian Wildlife Health Centre | -37.682651 | 145.535177 | Victoria |
| Adelaide Koala & Wildlife Centre | -34.817141 | 138.558197 | South Australia |
| Kangaroo Island Koala and Wildlife Rescue Centre | -35.786604 | 137.22901 | South Australia |

**References**

1. Howell, L.G.; Frankham, R.; Rodger, J.C.; Witt, R.R.; Clulow, S.; Upton, R.M.O.; Clulow, J. Integrating biobanking minimises inbreeding and produces significant cost benefits for a threatened frog captive breeding programme. *Conserv. Lett.* **2021**, *14*, e12776, doi:https://doi.org/10.1111/conl.12776.
2. Howell, L.G.; Mawson, P.R.; Frankham, R.; Rodger, J.C.; Upton, R.M.O.; Witt, R.R.; Calatayud, N.E.; Clulow, S.; Clulow, J. In-tegrating biobanking could produce significant cost benefits and minimise inbreeding for Australian amphibian captive breeding programs. *Reprod. Fertil. Dev.* **2021**, *33*, 573–587.
3. Howell, L.G.; Mawson, P.R.; Comizzoli, P.; Witt, R.R.; Frankham, R.; Clulow, S.; O'Brien, J.K.; Clulow, J.; Rodger, J.C. Modelling genetic benefits and financial costs of integrating biobanking into the conservation breeding of managed marsupials. *Conserv. Biol. In Review.*
4. McLean, N.; Handasyde, K.A. Sexual maturity, factors affecting the breeding season and breeding in consecutive

seasons in populations of overabundant Victorian koalas (*Phascolarctos cinereus*). *Aust. J. Zool.* **2007**, *54*, 385–392.

5. Johnson, R.N.; O'Meally, D.; Chen, Z.; Etherington, G.J.; Ho, S.Y.W.; Nash, W.J.; Grueber, C.E.; Cheng, Y.; Whittington, C.M.; Dennison, S. Adaptation and conservation insights from the koala genome. *Nat. Genet.* **2018**, *50*, 1102–1111.